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November 29, 2011

Eileen Maher
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Dear Ms. Maher,

Please find attached the final report for our project “Eelgrass in San Diego Bay: Assessing Eelgrass Habitat Function for Recreationally Important Species”. Our report contains the rationale and findings for several projects that took place between 2008 and 2011, each of which contributed to our overall goal of assessing how the structure of San Diego Bay eelgrass habitat influences predator-prey interactions, biodiversity of eelgrass epifauna, and nursery habitat function for juvenile fishes. Funding from the Port of San Diego for these projects allowed us to make significant strides in the study of eelgrass habitat function; to educate children about the value of marine habitats; and also allowed a variety of San Diego State graduate and undergraduate students to complete theses, conduct independent study, produce peer-reviewed publications, and make presentations at scientific conferences.

We look forward to continuing to work with the Port of San Diego on projects in San Diego Bay.

Sincerely,

Dr. Kevin A. Hovel
Department of Biology
San Diego State University

Eelgrass in San Diego Bay: Assessing Eelgrass Habitat Function for Recreationally Important Species



Final Report

Assembled for the San Diego Unified Port District

November 29, 2011

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1. Summary

In San Diego Bay (SDB), eelgrass (*Zostera marina*) serves as a habitat for a variety of fishes and invertebrates such as juvenile giant kelp fish, barred and spotted sand bass, calico bass, and spiny lobster. Eelgrass may serve as an important refuge for these species, particularly in their juvenile stages, and eelgrass houses many small invertebrates that provide them with food. Despite the widely cited function of eelgrass as a nursery habitat for species such as these, critical information regarding the function of this habitat is missing. In this project we conducted field experiments in SDB and lab experiments in the new Coastal and Marine Institute Laboratory (CMIL) that were focused on the effects of eelgrass habitat structure on ecological relationships involving juvenile recreationally important fishes. Our overarching goal was to provide a more complete understanding of how eelgrass functions as a foraging and refuge habitat for organisms in SDB. Specifically, we sought to determine how eelgrass structural complexity (i.e. the density and biomass of eelgrass shoots) varies within eelgrass patches in SDB and how this variation may influence the distribution, abundance, and behavior of recreationally important fishes and their invertebrate prey. Our specific objectives, and results-in-brief, were as follows:

Objective 1: Complete a field experiment in San Diego Bay that examines how eelgrass habitat characteristics interact with predation to dictate the abundance and distribution of eelgrass-dependent epifaunal species.

In 2008-2009 we conducted sampling and a manipulative experiment in the eelgrass habitat of the northern and central ecoregions of SDB. We tested how structural complexity varied within eelgrass patches and whether epifaunal abundance varied primarily with structural complexity, distance from patch edges, or predation pressure. Field sampling revealed that eelgrass structural complexity tended to increase from the edge of patches to the interior; however, epifaunal abundance depended on distance from the edge for some species (e.g. amphipods) and on structural complexity for others (e.g. grass shrimp). In a manipulative experiment, structural complexity generally had a larger influence on epifaunal abundance than did predation and distance from the patch edge, but predation was a very important factor for some key prey species such as grass shrimp.

Objective 2: Complete laboratory experiments focused on the effects of eelgrass habitat structure on organismal behavior, prey survival, and the foraging efficiency of juvenile fishes.

In separate studies from 2008-2011, we (i) tested whether habitat preference in fishes and invertebrates depends on eelgrass structural complexity and whether these preferences are influenced by risk of predation and food levels, and (ii) tested how eelgrass structural complexity influenced the foraging efficiency of juvenile fishes. The habitat preference experiments revealed that juvenile fishes strongly prefer highly complex eelgrass over low complexity eelgrass, even when we placed a predatory threat within high complexity eelgrass. In contrast, grass shrimp displayed only a weak preference for high complexity eelgrass. The experiments

on fish foraging efficiency revealed that relationships between eelgrass structure and the success of juvenile fishes in finding and capturing prey are not straightforward. Rather, the success of juvenile fishes in consuming prey sometimes decreased and sometimes increased as we increased eelgrass habitat structure within tanks, and the abundance of prey within the aquaria often had just as large an influence on the success of juvenile fishes as did eelgrass habitat structure.

Objective 3: Build a mathematical model using information gleaned from field experiments that predicts how eelgrass loss in SDB may influence predator-prey relationships.

Using NetLogo software, we constructed an individual-based computer model that simulates prey and predator organisms interacting within a simulated eelgrass landscape. The model user is able to vary the structure of the landscape (eelgrass structural complexity and patchiness) as well as the abundance and behaviors of the simulated fishes and invertebrates inhabiting the eelgrass. As the field experiments revealed major differences in epifaunal abundance and survival between patch edges and interiors, as well as with eelgrass structural complexity, a major goal of the model was to determine how habitat preferences dictate the success of mesopredatory fishes in capturing prey and surviving predatory attacks, both of which are crucial functions of eelgrass nursery habitats. Overall, the results suggest that the optimal nursery habitat exists when prey are abundant along patch edges and when juvenile fishes prefer patch edges, but only when the eelgrass landscape consists of continuous, high density eelgrass. Under this scenario, fishes can forage in low complexity patch edges, but retreat to high density patch interiors when predatory threats emerge. Fragmenting eelgrass into smaller, isolated patches tends to reduce the success of juvenile fishes, but interestingly, the model predicts that their success can be high when eelgrass is fragmented and structural complexity is low, but only when prey are densest in patch interiors. In this case, juvenile fishes can find prey relatively easily in low complexity eelgrass but also have moderate refuge from predators when they stay within patch interiors where prey are located.

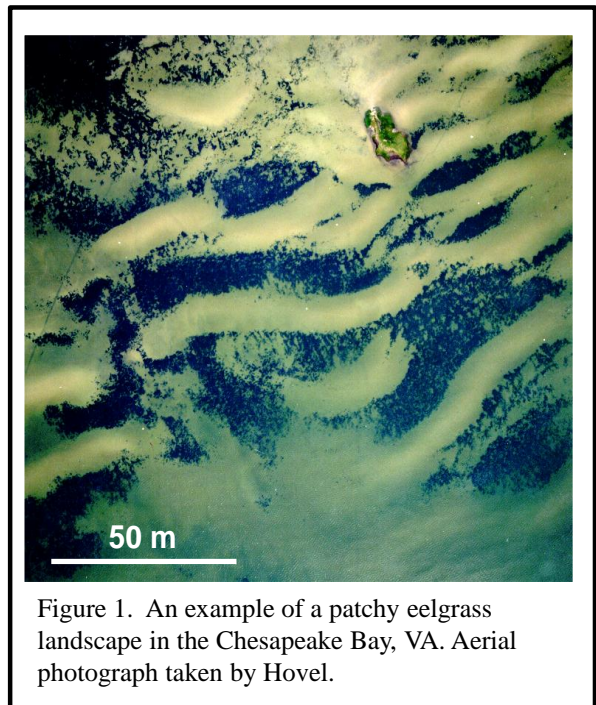
Objective 4: Conduct an outreach program in which SDSU graduate students and faculty instruct K-12 students about the value of SDB habitats for marine species and the value of using mathematical tools to study marine animals.

We constructed a simplified version of our eelgrass predator-prey model and brought K-12 students in the Ocean Discovery Institute's marine biology afterschool program to the SDSU campus for lectures and computer-based exercises led by SDSU faculty (PIs Hovel and Regan) and graduate students. Presentations focused on the value of modeling in science as an experimental tool, as well as on the value of eelgrass habitat for coastal marine species. The exercises involved students writing their own short programs in NetLogo (which illustrated how commands in NetLogo software create a simulated landscape in which organisms can exhibit programmed behaviors) and students using our simplified model to test hypotheses about the effects of eelgrass habitat structure on fish survival and foraging.

2. Introduction

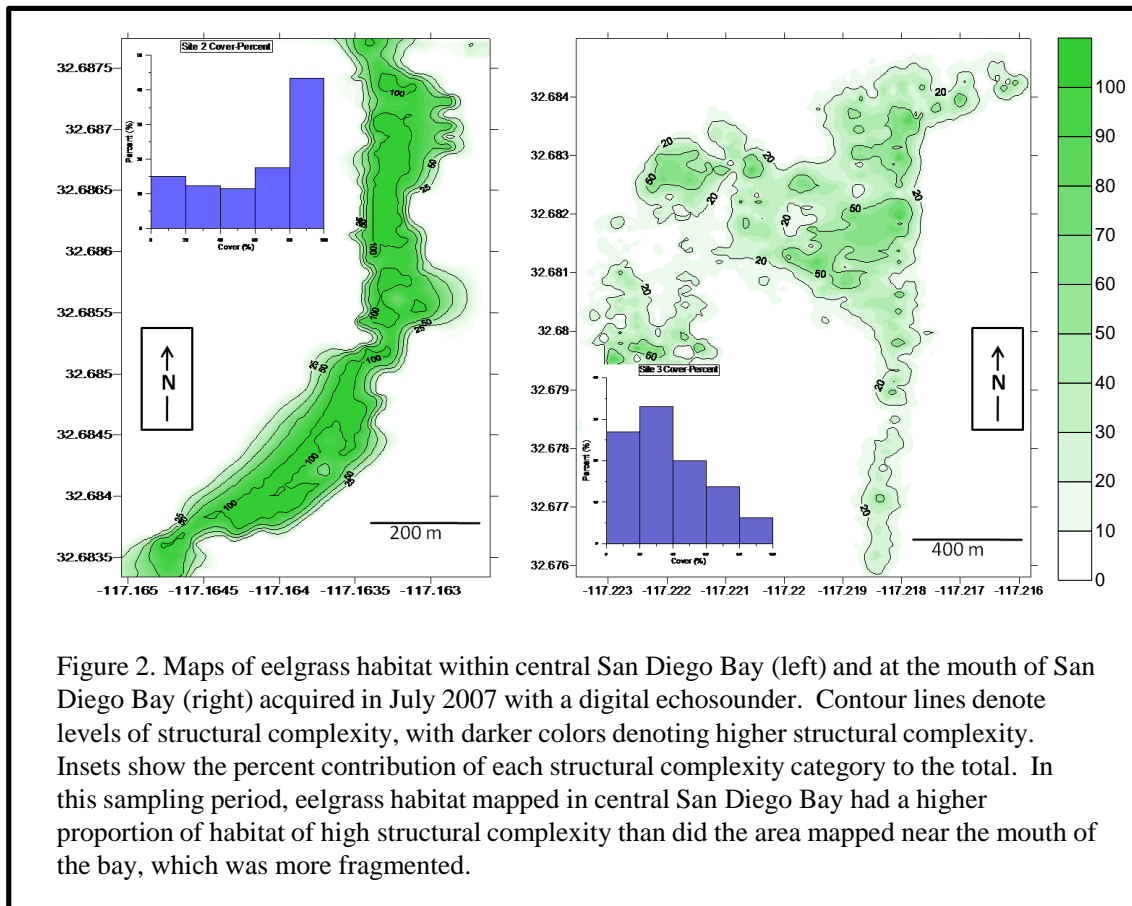
Eelgrass is one of several types of structurally complex marine habitats that house a diverse suite of interacting epifaunal and infaunal species and that may function as **nursery habitats** in coastal marine waters. Beck et al. (2001) identify marine nursery habitats as those with larger than average contributions, per unit area, to the production of individuals that recruit to the adult population. This may happen via combinations of enhanced density, growth, survival, and movement of individuals from a nursery habitat. Along with habitats such as kelp forests, oyster and rocky reefs, and coral reefs, eelgrass creates foraging habitat and refuge for a great variety of vertebrate and invertebrate organisms. Many of these organisms spend a significant portion of their life cycle in eelgrass habitat, from the time of settlement through their juvenile stages or beyond, where they take advantage of a diverse suite of prey and the refuge value of dense above-ground and below-ground biomass. Because eelgrass is widespread within the world's estuaries and along coastlines, it serves as one of the most critical essential fish habitats globally (Beck et al. 2001). Many of the organisms inhabiting eelgrass during the early portion of their life cycle are some of the most economically valuable fishery species in the oceans.

Many organisms, such as a variety of fishes, preferentially settle in eelgrass as larvae and experience high rates of survival and growth in eelgrass habitat. Organisms such as juvenile fishes can be considered **mesopredators** while inhabiting eelgrass, whereby they hunt for epifaunal or infaunal prey, but must avoid being eaten by higher-order predators. In turn, eelgrass habitat structure is one of the primary factors influencing refuge value and foraging success of these mesopredators (Heck and Crowder 1991, Orth 1992). Within a physically complex habitat such as eelgrass, variability in habitat structure can be observed in differences from patch-to-patch in *structural complexity*, which commonly is measured as the density or biomass of shoots (the above-ground material) and the biomass of roots and rhizomes (the below-ground material). Habitat structure also can vary at the *landscape scale* as the size of patches and how patches are distributed within the landscape (e.g., patchy vs. continuous configurations; **Figure 1**) which may be the product of natural and human-made disturbances. Habitat structure strongly influences ecological patterns, including the abundance, distribution, and diversity of animals, as well as the ecological processes that lead to these patterns (e.g. predation, growth,



reproduction, and competition) (Heck and Crowder 1991, Orth 1992, Attrill et al. 2000). For example, increases in shoot density and shoot biomass tend to increase survival of prey, because at the scale of individual organisms, the ability of predators to find and capture their prey often is inhibited by eelgrass blades (Heck and Crowder 1991). Additionally, eelgrass patchiness may, depending on the type of predator, make it easier or more difficult for predators to find prey, or make it more dangerous for small organisms (which often serve as prey for fishes) to disperse through the landscape (Irlandi 1994, Hovel and Lipcius 2001).

Despite decades of studies on the effects of eelgrass habitat structure on ecological processes at local scales, and more recent advances in our knowledge of how eelgrass landscape structure influences these processes, almost nothing is known about how organisms respond to structure at these two scales simultaneously. For instance, experiments with tethered and sedentary animals have shown that relative survival may be positively (Hovel and Lipcius 2001, Hovel and Fonseca 2005) or negatively (Irlandi 1994) influenced by fragmentation and patchiness, and as in terrestrial systems, ecological patterns and processes differ between the edge and the interior of patches (Bologna and Heck 1999, Tanner 2003). But covariation in structure at multiple scales is prevalent in eelgrass habitat: patches of different sizes (Hovel and Lipcius 2002), and patch edges and interiors, typically vary in structural complexity (**Figure 2**). Studies have either controlled for variability in structural complexity among patch sizes or



patchiness regimes (e.g. Hovel and Lipcius 2001), or have avoided fragmented eelgrass when testing how structural complexity influences ecological patterns and processes.

Fundamentally, variability in habitat structure influences the density and survival of organisms by influencing their behavior as they avoid their predators and search for prey (Stoner 1979, Holbrook and Schmitt 1988, Gotceitas 1990). Almost nothing is known, however, about the way in which habitat structure, particularly at multiple scales, influences the behavior of eelgrass fauna. In eelgrass and other marine habitats, most study organisms are sedentary or tethered in place to evaluate their relative survival among treatments. Tethering mobile organisms can reveal a great deal about their relative vulnerability to predators, and tethering often is one of the only ways in which relative vulnerability among treatments can be assessed under realistic environmental conditions. But preventing prey from exhibiting predator avoidance and other types of behaviors precludes examination of the effects of habitat structure on true outcomes of predator-prey interactions, and may bias estimates of relative vulnerability among treatments (Hovel and Regan 2007). For instance, prey and mesopredator responses to approaching predators, such as whether to hide or to swim away, may differ with structural complexity (Main 1987) or among patch sizes or with patch connectivity. Additionally, organisms may preferentially inhabit particular regions within an eelgrass landscape, such as patch edges, patch interiors, or areas of high structural complexity (Bologna and Heck 1999) and have adaptive behaviors there that are not exhibited or effective when they are placed elsewhere.

Thus, to understand how habitat structure influences ecological processes that dictate the abundance and distribution of eelgrass organisms – and ultimately the value of eelgrass as a nursery habitat – we must know how eelgrass habitat structure varies at multiple spatial scales and how variability in structure influences decisions animals make about foraging and avoiding predators. This is particularly true for juvenile fishes and invertebrates that have no size refuge from predation and depend on eelgrass habitat for both predator avoidance and foraging opportunities. For these mesopredatory species that may (via predation) strongly influence eelgrass growth, community structure, and primary production (Douglass et al. 2007), strategies for minimizing risk and maximizing prey intake may vary greatly among different combinations of structural complexity and landscape structure. Moreover, trade-offs in behavior may exist between maximizing prey capture and minimizing predation risk.

In this study, we conducted several experiments to address some fundamental questions regarding the role of eelgrass habitat structure in ecological interactions among organisms, with a specific focus on the ways in which habitat structure influences the ability of eelgrass to serve as a nursery habitat for mesopredatory juvenile fishes. This required establishing patterns of habitat structure and organismal distribution in naturally occurring eelgrass habitat in San Diego Bay, and then testing how habitat structure influences the decisions made by fishes and their invertebrate prey when in eelgrass habitat. Based on results from our experiments and information from the literature, we then developed a mathematical model describing how eelgrass habitat structure influences nursery habitat function.

3. Objectives

Our specific objectives were to:

- Complete a field experiment in San Diego Bay that examine how eelgrass habitat characteristics interact with predation to dictate the abundance and distribution of eelgrass-dependent epifaunal species;
- Complete laboratory experiments focused on the effects of eelgrass habitat structure on organismal behavior, prey survival, and the foraging efficiency of juvenile fishes;
- Build a mathematical model using information gleaned from field experiments that predicts how eelgrass loss in SDB may influence predator-prey relationships; and,
- Conduct an outreach program in which graduate students and faculty instruct K-12 students about the value of SDB habitats for marine species and the value of using mathematical tools to study marine animals.

4. Part A: field experiments on eelgrass epifauna

Rationale

In eelgrass habitat, structural variation at the patch- and landscape-scale both influence faunal distributions and predator-prey interactions, but their relative effects have rarely been tested simultaneously. The goal of this component was to address the relative influences of habitat structure at the patch- and landscape-scale on predator-prey dynamics and the distribution of epifaunal (prey) organisms as well as fishes (predators) within eelgrass habitat in SDB. We specifically tested the hypothesis that the distribution of epifaunal organisms would be dictated jointly by eelgrass structural complexity, distance from the patch edge, and predation pressure by fishes. We also tested the hypothesis that juvenile fishes would display a preference for patch edges or interiors, i.e., that their distribution would be non-random within eelgrass patches.

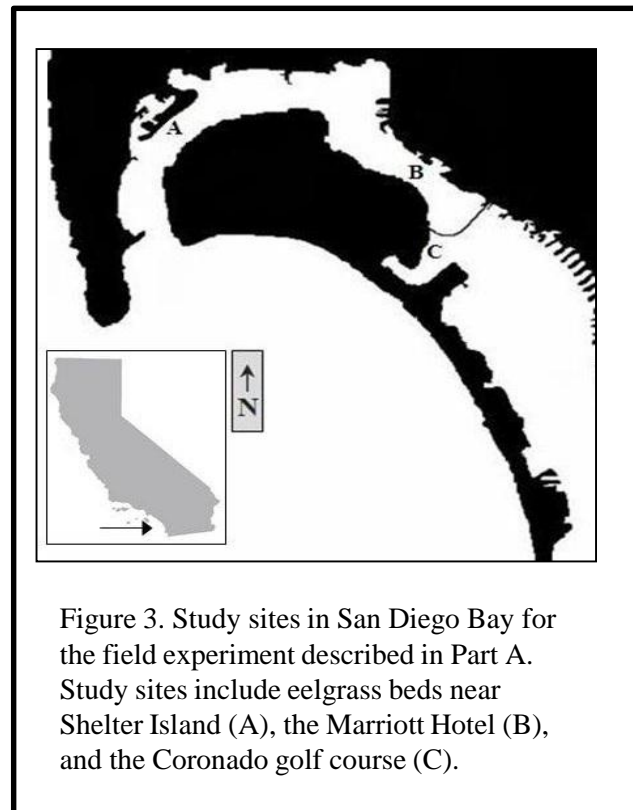


Figure 3. Study sites in San Diego Bay for the field experiment described in Part A. Study sites include eelgrass beds near Shelter Island (A), the Marriott Hotel (B), and the Coronado golf course (C).

Methods-in-brief

We conducted a field experiment between spring 2008 - fall 2009 examining how eelgrass habitat structure varies within eelgrass patches in SDB and how variation in eelgrass habitat structure influences the distribution, abundance, and diversity of eelgrass invertebrates and juvenile fishes. We sampled within the edge (both outer and inner edge) and interior of eelgrass beds in three locations in SDB (Shelter Island, by the Marriot Hotel in Coronado, and by the golf course in Coronado; **Figure 3**) for eelgrass habitat structure, fish abundance and diversity, and the abundance and diversity of invertebrate epifauna, including organisms such as amphipods, grass shrimp, isopods, and snails. Surveys were performed in June and in August of 2008. Fish abundance and diversity was measured by trawling with nets behind a small boat, and invertebrate abundance and diversity and eelgrass habitat structure were measured using eelgrass coring devices that sample small discrete areas of the edge and interior of the beds. In an accompanying field experiment we used artificial seagrass units (ASUs) to create our own small areas of simulated eelgrass within the edge and interior of patches, and controlled fish (predator) access to a subset of these ASUs using cages made of PVC pipe and plastic mesh (**Figure 4**). ASUs, which are readily colonized by epifauna, were used to control the structural complexity of small areas of the eelgrass bed. This resulted in a set of treatments allowing us to determine the relative role of structural complexity, distance from the patch edge, and predation on epifaunal abundance and diversity.

Results

Eelgrass structural complexity. Though there was some variability among the sites and survey periods, in general eelgrass habitat structure increases from the edge to the interior of beds (**Figure 5**). The two sites in the central ecoregion showed this pattern more consistently than did the eelgrass bed near Shelter Island in the northern ecoregion.

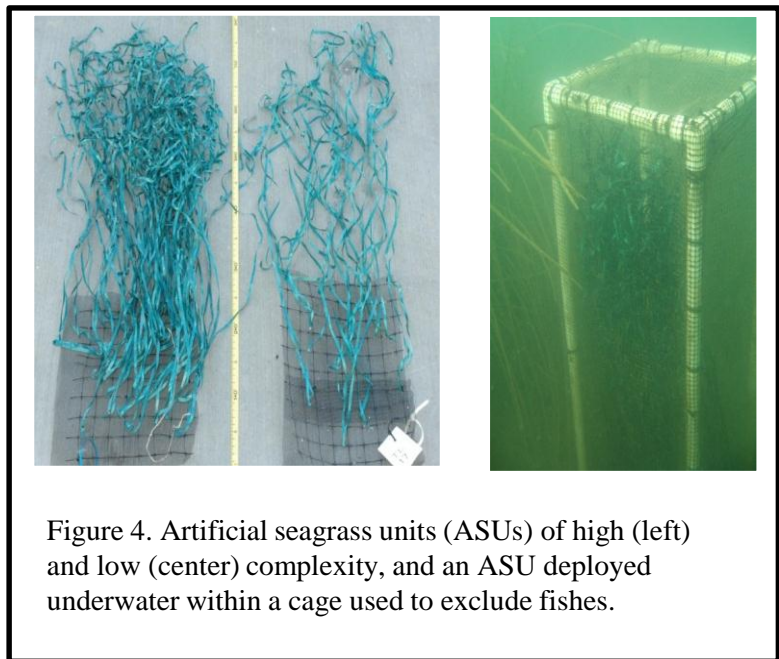


Figure 4. Artificial seagrass units (ASUs) of high (left) and low (center) complexity, and an ASU deployed underwater within a cage used to exclude fishes.

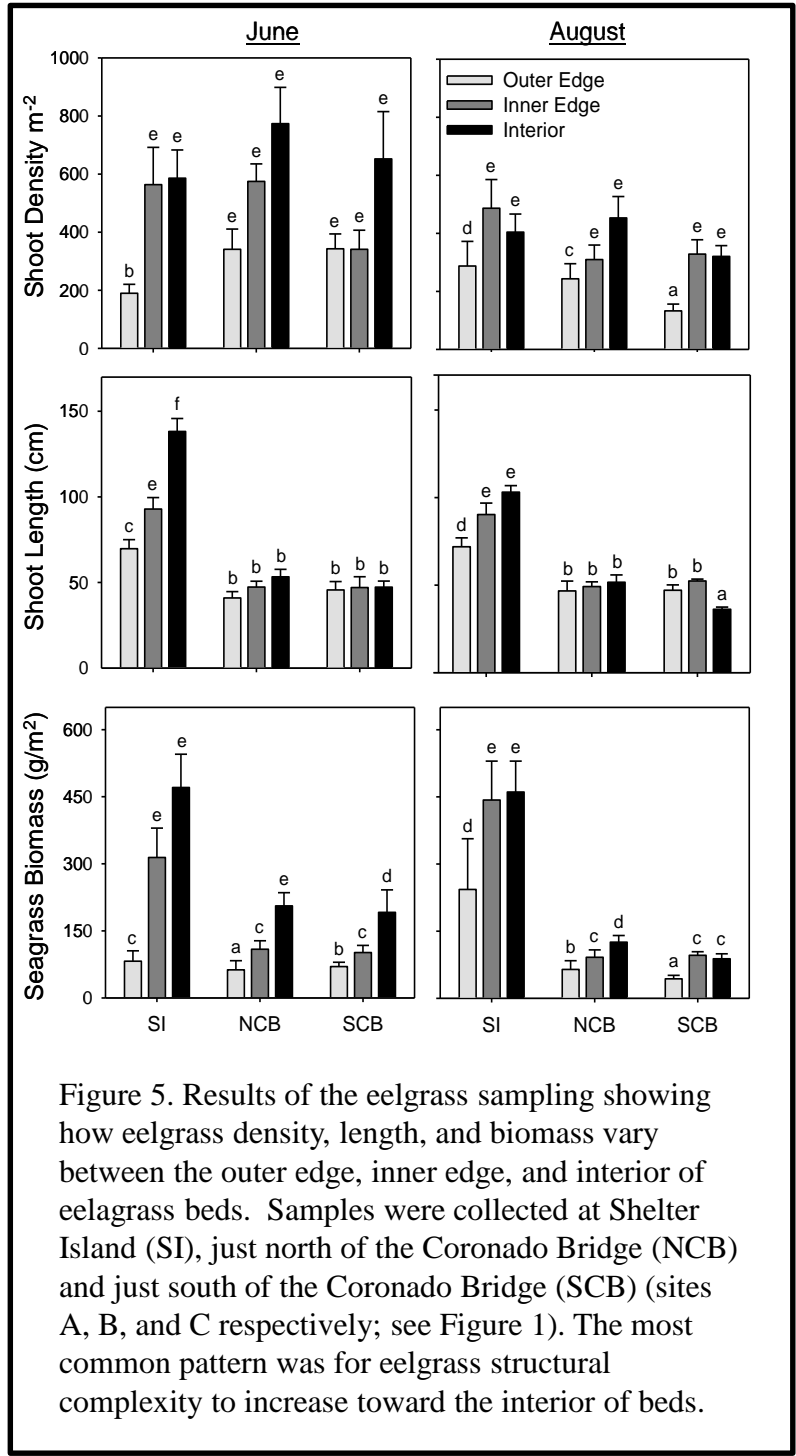
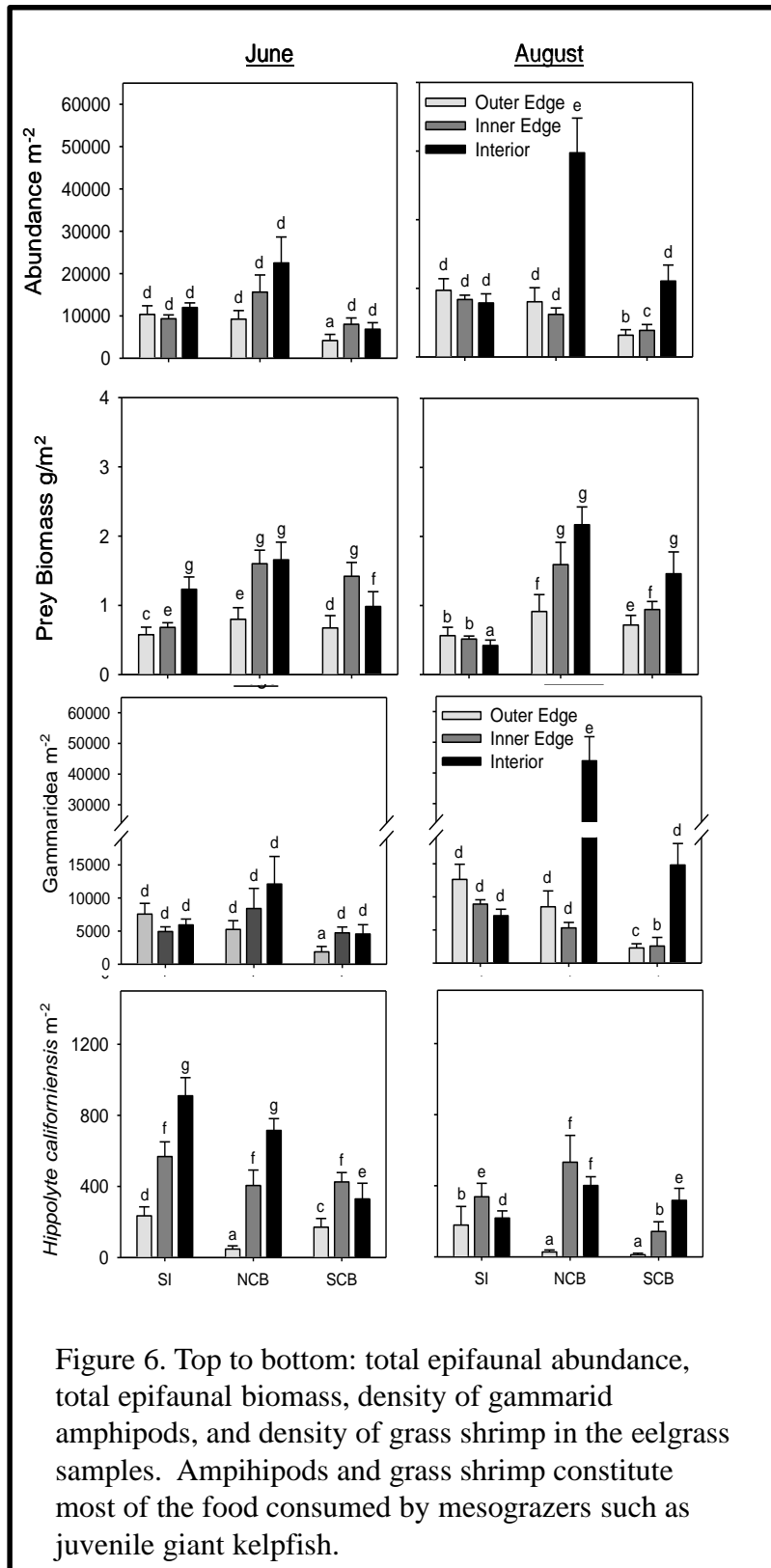


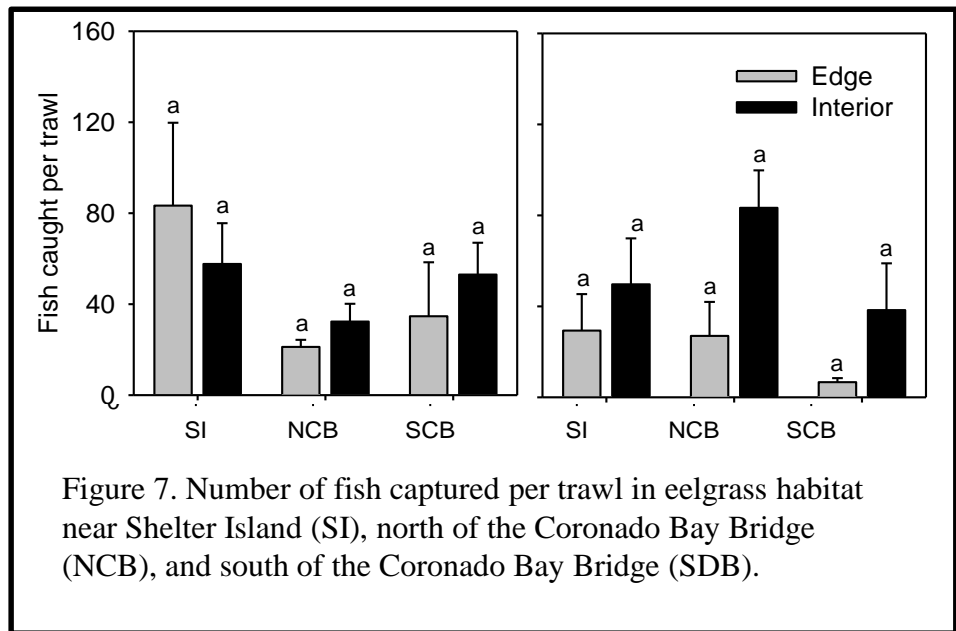
Figure 5. Results of the eelgrass sampling showing how eelgrass density, length, and biomass vary between the outer edge, inner edge, and interior of eelgrass beds. Samples were collected at Shelter Island (SI), just north of the Coronado Bridge (NCB) and just south of the Coronado Bridge (SCB) (sites A, B, and C respectively; see Figure 1). The most common pattern was for eelgrass structural complexity to increase toward the interior of beds.



Epifaunal abundance and distribution. Over the two survey periods approximately 60,000 epifaunal organisms were counted and sorted into a total of 46 taxonomic groups. The most abundant taxon, gammarid amphipods, comprised 56%, and 76% of total epifauna in June and August respectively. We found that many epifaunal invertebrates prefer dense interior eelgrass (**Figure 6**). Overall, eelgrass complexity (e.g. the density of eelgrass shoots, or relative biomass of shoots) was the overriding factor in dictating where most small invertebrate prey organisms would be found. However, variation in this trend was prominent, with different species having different preferences. Notably, amphipods, a major food source for juvenile fishes, often preferred the edges of eelgrass beds even though levels of eelgrass structure are reduced there. We speculate that this preference for edges exists because the delivery of planktonic food is greater along patch edges than within patch interiors.

Fish surveys. The abundance of fishes was slightly higher in patch interiors than at edges but this effect explained less than 10% of the total variation in the data (**Figure 7**). Thus, overall, there were no strong patterns of distribution for fishes. Fish abundance also was not strongly related to sampling period or site. We also examined the stomach contents of 191 fishes (**Table 1**). Gammarid amphipods made up the majority of the contents by abundance for all fish groups except pipefish, whose stomachs primarily contained copepods, ostracods, or cumaceans. Overall, there were no strong differences in fish diets between the edge and the interior.

Manipulative field experiment. Nearly 45,000 individual epifauna were collected from ASUs in the caging experiment and were sorted into 42 taxonomic groups. As with the epifaunal surveys, gammarid amphipods were the most abundant taxon (73% of individuals). Overall, distance from the edge and complexity had stronger effects on the epifaunal community than did predator access, with distance from the edge accounting for more variability than structural complexity (**Figure 8**).



Collectively, epifauna were more abundant at the patch edge than at the interior, regardless of structural complexity, and were more abundant in dense ASUs than in sparse ASUs, regardless of distance from the edge. Notably, grass shrimp (*Hippolyte californiensis*), which unlike burrowing ghost shrimp are small, green, canopy-dwelling crustaceans, were far more abundant in caged (predator-excluded) ASUs than in open and cage-control ASUs, and slightly more abundant in dense than in sparse ASUs, suggesting that they are strongly controlled by predation by fishes and that they prefer dense eelgrass over sparse eelgrass. Grass shrimp are a primary food source for some key juvenile fishes (e.g. giant kelpfish) and we used this species for our laboratory experiments due to its importance in the diet for fishes.

Table 1: Percent composition of fish stomach content analyses by abundance. COC = copepods, ostracods, and cumaceans. Molluscs include bivalves and gastropods, and worms include polychaetes and nematodes. All values greater or equal to 1% of the average stomach contents for each group are in bold.

| Fish group | N | Fork length, mm (SE) | Gammarid amphipods | Other peracarids | Shrimp | Crabs | COC | Molluscs | Worms | Fish |
|------------|----|----------------------|--------------------|------------------|-------------|-------------|--------------|-------------|-------------|------|
| Bass | 34 | 146.74 (9.05) | 56.9% | 24.2% | 6.2% | 1.5% | 0.8% | 9.0% | 1.2% | 0.1% |
| Kelpfish | 74 | 100.49 (3.28) | 89.8% | 6.0% | 3.3% | 0.0% | 0.5% | 0.1% | 0.0% | 0.2% |
| Perch | 39 | 75.82 (4.48) | 56.8% | 9.0% | 0.6% | 0.1% | 31.8% | 1.4% | 0.2% | 0.0% |
| Pipefish | 17 | 137.06 (10.26) | 33.9% | 0.6% | 3.7% | 0.0% | 61.9% | 0.0% | 0.0% | 0.0% |
| Other | 27 | 94.30 (5.36) | 93.1% | 4.3% | 0.7% | 0.1% | 0.8% | 0.2% | 0.7% | 0.0% |

Discussion

This study is the first to experimentally eliminate covariation between eelgrass local-scale structural complexity and proximity to patch edges to isolate the relative and interactive effects of these two scales of structure on epifaunal communities. Overall, our results indicate that local-scale and larger, patch-scale attributes of eelgrass habitat structure covary in SDB and jointly influence epifaunal and fish density, diversity, and community composition, and that the interactive effects of these factors differ among sites and sampling periods.

Because epifaunal densities often are higher at the edge of eelgrass patches than within the interior (e.g., Bologna and Heck 2002), eelgrass patch edges may be critical areas for transfer of secondary production to higher trophic levels. Though we found that taxa comprising a large portion of the diet of predatory fishes (e.g., amphipods) were more abundant along eelgrass patch edges than within interiors, we found little evidence that trophic transfer is higher along eelgrass patch edges at our sites (i.e., no interactive effect of predator access and proximity to edges on epifaunal abundance in our manipulative experiment). Likewise, we did not detect major

differences in fish (predator) abundance or diet between the patch edge and interior. Fishes captured in our beam trawl are vulnerable to predation by larger fishes and birds and may have to balance prey capture success, which may be higher in less complex habitat at the edge, with seeking refuge from their own predators by spending less time at the patch edge. A recent review of patterns of nekton abundance in seagrass (eelgrass and other species) also reported little evidence that fishes display distinct patterns of distribution throughout patches (Connolly and Hindell 2006). An exception in our study was that juvenile giant kelpfish, a cryptic species in eelgrass habitat, were consistently more abundant in patch interiors than near edges. This species can often be seen drifting, hanging vertically in the water where they become camouflaged among the eelgrass blades, and likely utilize the interior of the patch for concealment.

Our manipulative experiment allowed us to examine the relative effects of distance from the edge, eelgrass structural complexity, and predator access on the epifaunal community. For many taxa (e.g., gammarid and caprellid amphipods, tanaids), distance from the edge accounted for more of the variability among plots than did structural complexity or predator access. We found few differences between sparse and dense ASUs along the edge, suggesting that epifauna may preferentially occur at that location, regardless of structural complexity variability within the patch (see also Tanner 2005). Several mechanisms may account for this. Within eelgrass systems, organisms dispersing among patches may remain within the habitat they first encounter (the “nearest refuge” hypothesis: Virnstein and Curran 1986, Tanner 2005) leading to higher abundance along patch edges than in patch interiors. Similarly, when dispersing larvae in the water column encounter the structure of an eelgrass bed, they may “settle and stay” regardless of microhabitat characteristics, thereby also leading to higher faunal abundance at the patch edge (Bologna and Heck 2002). In addition to movement and dispersal behavior, the feeding mode of an

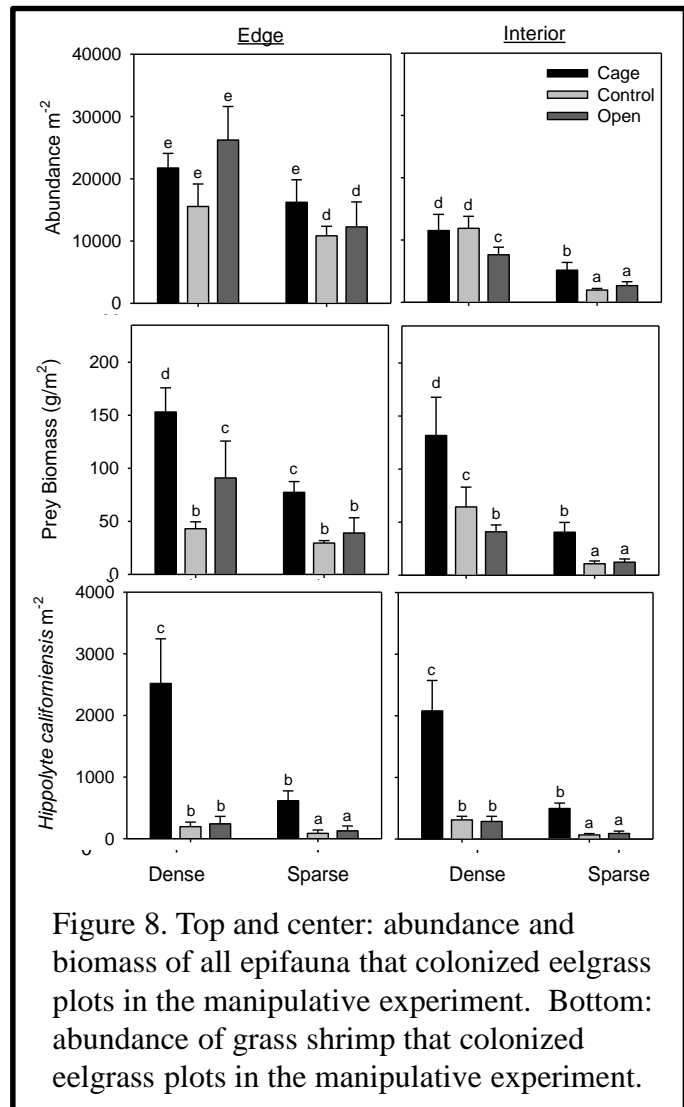


Figure 8. Top and center: abundance and biomass of all epifauna that colonized eelgrass plots in the manipulative experiment. Bottom: abundance of grass shrimp that colonized eelgrass plots in the manipulative experiment.

organism likely influences its abundance at habitat edges. Many marine invertebrates that rely on currents to deliver planktonic food may exhibit higher abundance or growth along patch edges where currents are typically stronger (Bologna and Heck 1999, Peterson et al. 2004). In seagrass habitat in St. Joseph Bay, Florida, epifaunal density was higher along patch edges than interiors, despite the fact that seagrass biomass and shoot density were higher in patch interiors (Bologna and Heck 2002). Similarly, epifaunal density was higher at seagrass-sand edges than in seagrass patch interiors in South Australia, despite the fact that seagrass biomass per unit area increased from the edge to the interior (Tanner 2005). In our study, caprellid amphipods were typically more abundant along patch edges than in patch interiors, regardless of shoot density. These amphipods feed by sweeping the water column with enlarged gnathopods while grasping seagrass blades with modified abdominal appendages (Brusca and Brusca 2002) and they may benefit from greater food delivery to the canopy near the patch edge (Bologna and Heck 1999, Peterson et al. 2004).

Predator access strongly affected the abundance of the grass shrimp *H. californiensis*, which was well represented in predator stomachs. Grass shrimp were more abundant in patch interiors than at patch edges in our epifaunal survey, and results of our manipulative experiment suggest that these shrimp prefer patch interiors due to the protective function of dense eelgrass shoots found there. Although gammarid amphipods were the most abundant taxa in fish stomachs, their high abundances across caging treatments, and in surveys, suggest their distribution is not predator-limited.

Though all eelgrass patch edges bordered unvegetated sediment in our study, we found that effects of structural complexity and proximity to patch edges on fauna varied between sites within sampling periods, as well as between sampling periods. Relationships between habitat structure and epifaunal community structure differed strongly between our front-bay site (SI) and our two central bay sites (NCB and SCB) suggesting that estuarine-scale processes also influence the epifaunal communities within SDB. Gradients in tidal flux, water retention time, temperature, salinity, and other factors with distance from the bay mouth may influence larval delivery and community-level interactions as well as the size of the “ecological edge” of a patch (Largier et al. 1997). Additionally, recruitment pulses are common for many of the epifaunal species present in our samples (Bologna and Heck 2002, Sirota and Hovel 2006), which can lead to large fluctuations in epifaunal abundance between sampling periods. Moreover, the biomass and structure of the eelgrass itself changes seasonally; long reproductive shoots are produced in early summer, and blades become increasingly fouled by bryozoans and epiphytes from mid to late summer. It is important to note that our study did not include replicate patches within different regions in SDB, and that we therefore were unable to determine the primary causes of variability among sites. Experiments that include replicate patches within sites along an estuarine gradient (e.g., Jenkins et al. 1998) are necessary to determine how landscape context influences the effects of habitat structure at multiple spatial scales.

In summary, the results of our field surveys and experiment suggest that the organisms living in the eelgrass beds of San Diego Bay respond not only to local-scale, within-patch measures of habitat complexity but also to larger scale attributes of structure such as proximity to patch edges. We also found that relationships between epifaunal and eelgrass habitat structure vary by site, and that some taxa seemed to be strongly influenced by factors outside the scope of our study. Future research addressing faunal responses to variation in other aspects and scales of habitat structure will add to the growing knowledge of the environmental and ecological phenomena maintaining the diverse communities found in eelgrass beds and other ecosystems.

5. Part B: lab experiments on fish and invertebrate behavior

Rationale

In these experiments we tested how eelgrass habitat structure influences behaviors exhibited by fish and invertebrate (prey) species. All experiments involved two species that are abundant in eelgrass habitat, the epifaunal grass shrimp *Hippolyte californiensis* (a small, green, canopy-dwelling crustacean that is a major food source for juvenile fishes) and the mesopredatory juvenile giant kelpfish *Heterostichus rostratus*. We conducted two separate studies. The first study examined whether these two species actively select particular types of eelgrass habitat, as well as what factors may influence this selection, and the second study measured the influence of eelgrass structure on the foraging efficiency of juvenile fishes. For the first study, we hypothesized that fishes and invertebrates would select high structural complexity habitat over low structural complexity habitat, but that grass shrimp would be more selective than fishes, and that the addition of a predatory threat to high complexity eelgrass would result in a shift of preference to low complexity eelgrass. For the second study, we hypothesized that increasing eelgrass structural complexity would reduce the foraging efficiency of juvenile kelpfish, but that the level of prey density present in the experiment would modify this relationship. Both the abundance of prey and the amount of structural complexity may influence foraging success of fishes, but it is unknown how these factors interact, and which may be more important in determining how successful fishes are at capturing prey in eelgrass habitat.

Methods-in-brief

Study #1 involved monitoring the behavior of grass shrimp and juvenile kelp fish in large outdoor mesocosms (circular fiberglass tanks filled with seawater) in which the animals were allowed to choose between high complexity artificial eelgrass (on one side of the mesocosm) and low complexity artificial eelgrass (on the other side of the mesocosm). The distribution of animals within these “choice” treatments was compared to the distribution of animals in control “no choice” treatments (i.e. mesocosms containing only high complexity eelgrass or only low complexity eelgrass) to ensure that artifacts of the mesocosms themselves did not cause grass shrimp or kelpfish to select high or low complexity habitat. Experiments were performed separately for grass shrimp and for fishes. We created simulated eelgrass (known as artificial

seagrass units, or ASUs) by weaving green polypropylene curling ribbon to a plastic mesh base, which was buried under a layer of sand within mesocosms (**Figure 9**). In this manner we precisely controlled the amount of structural complexity on each side of the mesocosm. After placing shrimp or fishes into the center of mesocosms, we monitored their distribution (i.e. the proportion of individuals found within high density vs. low density eelgrass) over time. Grass shrimp were given 24 hours to acclimate and choose between the two habitat types, and juvenile kelpfish were monitored for one hour to calculate the proportion of time spent within both habitat types. This initial preference experiment was followed by an experiment in which a predatory threat was placed within the high complexity ASU (which generally was the preferred habitat) to determine whether habitat preference shifted to low complexity habitat. The predatory threat consisted of larger fishes (within cages so that they would remain on the appropriate side of the mesocosm). Likewise, food was added to low complexity eelgrass (in the absence of predators) to determine if that would motivate grass shrimp or kelpfish to spend more time in the initially less preferred habitat. Finally, for kelpfish only, a fourth experiment was run in which predators were added to high complexity habitat and food was added to low complexity habitat.

For study #2, we observed the foraging behaviors of juvenile kelpfish as they hunted grass shrimp prey within glass aquaria in which we added different amounts of eelgrass habitat (**Figure 9**). In this manner we were able to determine how specific behaviors varied in response to variation in eelgrass habitat structure. We conducted experiments within glass aquaria (122 x 61 x 61 cm) filled to a depth of 8 cm with clean beach sand and then filled with recirculating seawater to a depth of 45 cm. We varied structural complexity within aquaria by transplanting eelgrass from San Diego Bay to create six shoot densities, each randomly assigned to one aquarium: 20, 40, 80, 160, 250, or 320 shoots m^{-2} . To examine the relative effects of varying prey density and varying structural complexity on prey survival, encounter rates, foraging behavior of predators, and predator avoidance behavior by prey, we conducted two experiments, one in which grass shrimp density was held constant across the six levels of structural complexity, and one in which grass shrimp density increased with structural complexity. We used a density of 20 shrimp per mesocosm in the constant prey density experiment, and in the variable prey density experiment we increased shrimp density proportionally with shoot density to maintain an approximate ratio of one grass shrimp for every 2-3 shoots (Table 1). Fish behaviors observed included (i) the amount of time actively searching for prey, (ii) the ability to detect prey, (iii) the decision to attack prey once detected, (iv) the relative success of those attacks, and finally (v) the decision to chase after prey item if it was missed in the original attack.

Results

Study #1: habitat selection. Generally, grass shrimp and kelpfish preferred high complexity eelgrass over low complexity eelgrass, but trends differed between species and between experiments with and without predators. Grass shrimp exhibited relatively modest preference for high complexity eelgrass in mesocosms as well a tendency to change preference to

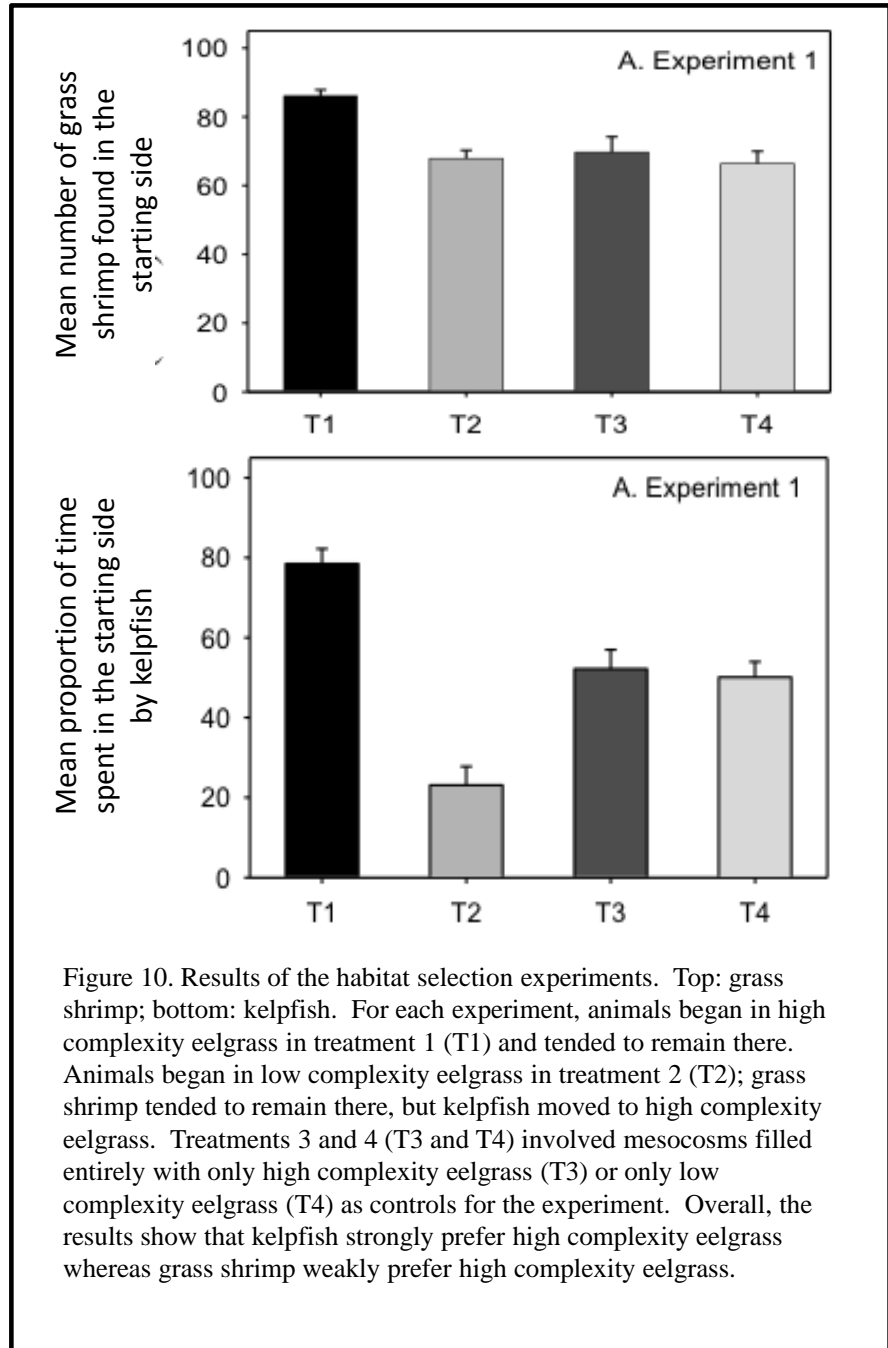


Figure 9. Photograph of (top) an outdoor, flow-through mesocosm containing artificial eelgrass in which habitat selection experiments took place, and (bottom) a glass aquarium containing transplanted eelgrass habitat in which fish predation trials took place.

low complexity eelgrass when a predatory threat was present in high complexity eelgrass (**Figure 10**). In contrast juvenile giant kelpfish exhibited a strong preference for high complexity eelgrass even when a predatory threat was present there and food was made available in low complexity eelgrass. Instead of moving to low complexity eelgrass habitat to escape from predators, juvenile kelpfish displayed antipredator behaviors where they remained motionless and vertical so as to hide within eelgrass leaves. This was surprising as we expected juvenile kelpfish to readily move away from predators even if this meant moving to habitat in which refuge value was reduced.

Study #2: kelpfish foraging efficiency. The experiments revealed that

variation in structural complexity and variation in prey density both strongly influence juvenile giant kelpfish hunting behavior. Specifically, the ability of kelpfish to detect prey was jointly influenced by structural complexity and prey density (**Figure 11**). However, the decision regarding whether or not to attack prey after detection depended only on prey density, and surprisingly, the proportional success of kelpfish in their attacks on prey depended on neither structural complexity nor prey density (**Figure 12**). Overall, our results show that prey density is



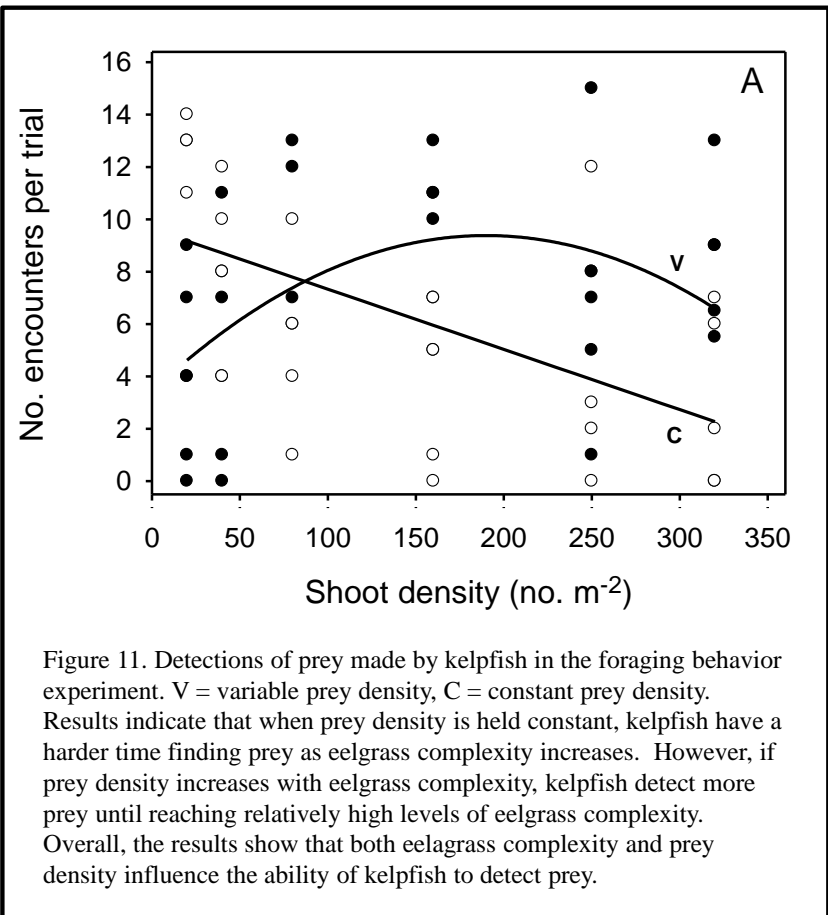
as important, or more important to consider when assessing how eelgrass habitat influences the foraging of juvenile fishes.

Discussion: Study #1: habitat selection

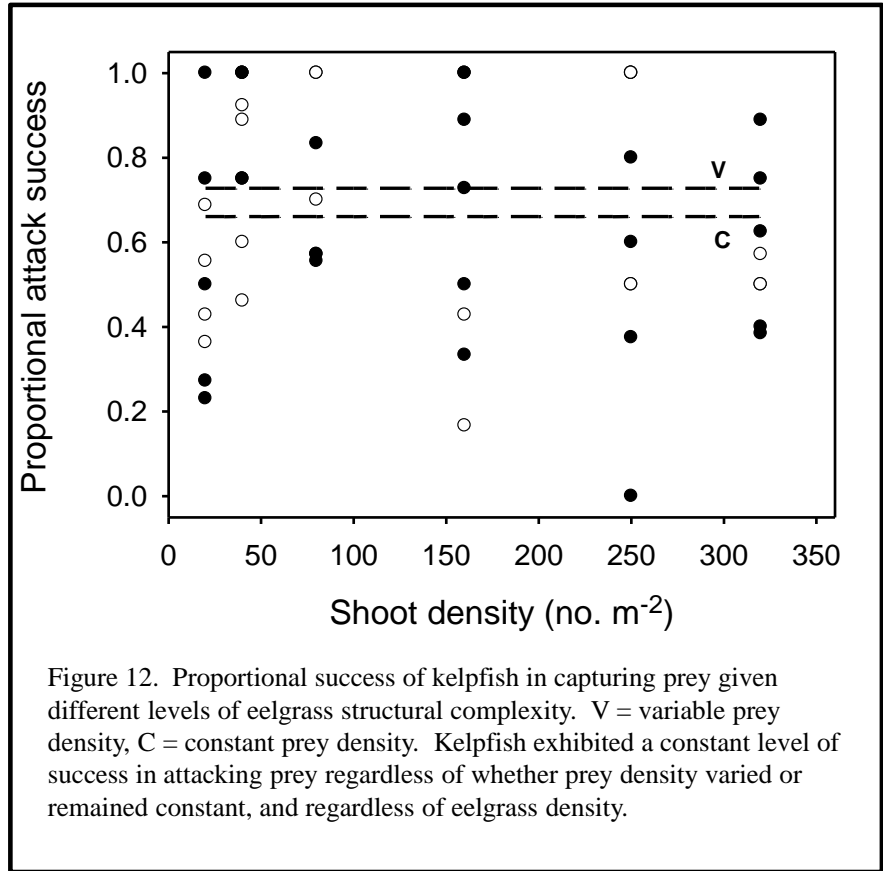
Though positive relationships between organismal abundance and seagrass structural complexity are common for aquatic epifaunal animals, the role of habitat selection (as opposed to differential mortality among habitat treatments) in structuring these relationships often is unknown, and few studies have truly quantified habitat preference through comparisons of choice vs. no choice treatments. Our primary conclusion from this study is that eelgrass structural complexity has a dominant effect on habitat selection at multiple trophic levels; specifically, kelpfish, and to a lesser extent grass shrimp, preferred high complexity eelgrass over low complexity eelgrass, even when predators were present in high complexity eelgrass or food was available only in low complexity eelgrass.

Though grass shrimp displayed only marginal preferences for habitat in laboratory mesocosms, in the field experiment for objective 1 their abundance was far higher within high complexity plots than in low complexity plots, regardless of whether predatory kelpfish were present in plots. Similarly, juvenile giant kelpfish preferred dense eelgrass that contained a predator over less complex eelgrass that contained food, even though kelpfish were starved for two days before the experiment began.

Our results suggest that both grass shrimp and kelpfish are able to differentiate among levels of eelgrass structural complexity and generally select more complex eelgrass habitat. For a variety of taxa, highly complex habitat may be preferred because of reductions in the ability of predators to find and capture their prey (Horinouchi et al. 2009). One surprising aspect of our results therefore was that epifaunal grass shrimp, which are commonly found in the guts of mesopredators such as juvenile giant kelpfish,



only displayed preference for high complexity eelgrass habitat in one out of three experiments. Weak trends for selection of high complexity eelgrass habitat could be due to the propensity of grass shrimp to settle onto simulated eelgrass blades immediately upon release. Though the number of grass shrimp remaining on the starting side of mesocosms was significantly higher when they began in high complexity vs. low complexity, over 50% of grass shrimp remained on the starting side of mesocosms in all treatments



(even in the absence of choice). Grass shrimp may choose to remain associated with the area of microhabitat that is first contacted, which provides some level of protection from potential predators, even if more suitable habitat might be found through exploration. Similarly, the microgastropod *Eatoniella atropurpurea* tended to remain within the starting segment of experimental mesocosms regardless of whether mesocosms contained a choice of habitats or no choice, though two other microgastropod species displayed clear preferences for habitats in which they are primarily found in nature (Olabarria et al. 2002). Though grass shrimp can detect and respond to predatory threats, there may be little incentive for them to search for better habitat unless they are approached or chased by a predator. Indeed, in study #2, we observed grass shrimp choosing to remain motionless on eelgrass blades unless a predatory attack was imminent. Additionally, *Tozeuma carolinense*, a species of grass shrimp similar to our study species, often switch side of eelgrass blades to avoid contact with approaching predators, rather than flee from them (Main 1987).

Due to the need for kelpfish to balance foraging and predator avoidance, we expected to find weaker trends for habitat preference for kelpfish than for grass shrimp. In contrast, mesopredatory juvenile giant kelpfish displayed a strong preference for high complexity eelgrass in three of the four experiments. This is similar to previous findings in which some mesopredatory species prefer higher complexity eelgrass habitat (Bell and Westoby 1986, Steffe

et al. 1989). Nonetheless it was surprising that kelpfish preferred high complexity eelgrass over low complexity eelgrass even when predators were present in high complexity eelgrass. A preference for habitat offering maximum refuge, even if predators are present there, has been observed for other species. Juvenile walleye pollock, *Theragra chalcogramma*, preferred unvegetated habitat over vegetated habitat in the absence of predators, but moved into vegetated habitat when predator mimics were placed into vegetated habitat (Sogard and Olla 1993). Juvenile bluegill sunfish (*Lepomis macrochirus*) selected high complexity habitat for increased cover from predators at the expense of foraging success (Gotceitas 1990), but also primarily inhabited complex artificial habitat rather than open habitat even when predators were present in the complex habitat (Savino and Stein 1989). In laboratory experiments Jordan et al. (1996) reported the crayfish (*Procambarus alleni*) not only exhibited preference for higher complexity structure, but also decreased their susceptibility to predation by doing so.

Though kelpfish preference for high complexity eelgrass habitat did not depend on predator presence, their swimming behavior did. In the absence of predators, kelpfish would spend a majority of their time in the high complexity portion of the mesocosm, making short forays into the less complex habitat before quickly returning to the high complexity side. Within the high complexity side, kelpfish would alternate between freely swimming within the dense eelgrass and occasionally orienting themselves vertically with ASU blades (an antipredator behavior associated with avoiding detection). With a predatory threat present in high complexity eelgrass, kelpfish movement was kept to a minimum with fish remaining vertically oriented with ASU blades, many for the entire study period. Similar to our results, crayfish (*Orconectes propinquus*) decreased active behaviors and increased defensive behaviors in the presence of predatory smallmouth bass (Stein and Magnuson 1976) and darters (*Etheostoma nigrum*) reduced activity levels in the presence of predators and maintained reduced activity levels for at least 24 h after predators were removed (Rahel and Stein 1988). We often observed fish swimming freely within high complexity eelgrass area but upon encountering the interface between dense and sparse eelgrass, fish would quickly turn back into high complexity eelgrass. When individuals did decide to cross into the less dense area of the mesocosm, many stopped at the interface before making forays into the less complex habitat, which were short and marked by constant swimming.

In summary, for study #1 we found both grass shrimp and kelpfish exhibited preferences for high complexity habitat, and that habitat preference and behaviors were modified by predator presence. We attribute preferences to the fact that each of our study organisms is vulnerable to higher-order predators that commonly inhabit southern California eelgrass habitat, though we were surprised by the lack of preference by grass shrimp, in some cases, for high complexity eelgrass. Our research highlights the importance of considering the covariation of several factors, as well as the importance of considering behavioral responses of individuals, when trying to predict habitat selection and species distributions in eelgrass beds.

Discussion: Study #2: kelpfish foraging efficiency

Though studies documenting relationships between prey survival and habitat structure are common in marine systems, relatively few studies have examined the specific mechanisms by which habitat structure influences predator-prey interactions (but see Ryer et al. 2004, Stoner 2009) and none that we are aware of have examined the relative effects of habitat structure and prey density on these mechanisms in structured marine habitats. There are several components to predator-prey interactions, each of which may be influenced by structural complexity and prey density: (i) prey detection by predators, (ii) decisions by predators to attack prey, (iii) predator success in capturing prey, and (iv) decisions by predators to pursue prey in the event they are unsuccessful. In addition, habitat structure and prey density may influence (v) the ability of prey to detect threats from predators and to take action to avoid attacks (Ryer et al. 2004). By examining each of these behavioral components, the mechanisms structuring relationships between prey survival (or conversely predator foraging success) and habitat structure can be elucidated.

Our results suggest that eelgrass structural complexity and prey density drive patterns of prey survival primarily through variability in rates of prey detection by predators, and that variability in prey density plays an important role in determining the likelihood that an individual prey organism encounters a predator. Though we found an inverse correlation between eelgrass structural complexity and encounter rates, encounter rates decreased quickly to a lower plateau when prey density increased with structural complexity, but decreased only slightly (though significantly) with structural complexity when prey density was held constant. Reduced predator-prey encounter rates in highly complex habitats commonly is caused by interference of structural elements with predator vision. For instance, largemouth bass (*Micropterus salmoides*) detection of prey (bluegill sunfish *Lepomis macrochirus*) decreased with increasing density of artificial plant stems, though differences in prey behavior among stem densities also influenced predation success (Savino & Stein 1982). Pipefish (*Syngnathus fuscus*) detection rates of amphipod prey were lower in structurally complex artificial eelgrass than in structural simple artificial eelgrass (Ryer 1988), and detection of *Daphnia pulex* by the topmouth gudgeon (*Pseudorasbora parva*) decreased with increasing densities of artificial macrophyte stems (Manatunge et al. 2000). In contrast, sponge habitat structure did not decrease detection rates of juvenile rock sole (*Lepidopsetta polyxstra*) and age-0 Pacific halibut (*Hippoglossus stenolepis*) by predatory age-2 Pacific halibut in laboratory experiments; rather, sponges served as a barrier to predator movement, thus interfering with predator search and capture (Ryer et al. 2004).

Prey behavior also may have influenced patterns of prey survival. There was a decrease in grass shrimp escape attempts with structural complexity that likely was due to reduced ability of grass shrimp to detect approaching and attacking predators. Though there were similar patterns between the two experiments, differences among levels of structural complexity in the ability of grass shrimp to detect predators appeared to be somewhat larger when prey density was held constant than when prey density varied. This suggests a decreased ability of grass shrimp to detect threats as structural complexity is increased. Though it is unclear why increasing prey

density with structural complexity would slightly modify this effect, one possibility is that grass shrimp observe more escape attempts from conspecifics when prey density is high, and therefore are more vigilant. Surprisingly, we did not observe changes in prey behaviors with habitat structure that minimize encounter rates with predators. We predicted that side switching by grass shrimp, which puts an opaque barrier between predator and prey, would be more frequent at low levels of structural complexity, but this was not observed. In contrast, a different species of grass shrimp from eelgrass habitat on the US east coast, *Tozeuma carolinense*, exhibited strong reaction to predators by moving around eelgrass blades and reducing time spent in motion (i.e. walking on blades; Main 1987).

Predator activity levels often decrease with habitat structure as predators switch from active searching to sit-and-pursue predation (Savino & Stein 1982, Ryer 1988, Michel & Adams 2009) which may help reduce the negative effects of structural complexity on foraging efficiency. Predatory beetle (*Dytiscus* spp.) larvae switched from actively foraging to a sit-and-pursue foraging strategy as structural complexity increased, resulting in similar rates of prey capture among levels of structural complexity (Michel & Adams 2009) as was true for lined seahorses (*Hippocampus erectus*) feeding on grass shrimp (*Hippolyte zostericola*) in simulated eelgrass habitat (James & Heck 1994). We did not find effects of eelgrass habitat structure on predator activity levels, though we observed a weak trend for kelpfish to decrease activity levels with increasing structure when prey density was held constant. This trend may have been stronger if we had used higher levels of structural complexity; in fact, in follow-up experiments using simulated eelgrass within mesocosms, kelpfish activity was significantly reduced at a higher shoot density (600 m⁻²) than used herein (K. Hovel, unpublished data).

In conclusion, for study #2 we demonstrated that prey density modifies the effects of eelgrass structural complexity on predation, and that different components of predator-prey interactions respond to variability in prey density in different ways. Caveats of our study include the fact that we used relatively low levels of eelgrass densities and prey densities in our experiments, as well as only one pair of predator-prey species. Our results should be extrapolated to other, denser eelgrass habitats and to other species cautiously, and more research is needed for other eelgrass habitats and species that may include higher levels of structural complexity and prey density. Our work also took place in a controlled laboratory setting in which organisms were not able to choose among habitats or levels of structural complexity. In naturally occurring eelgrass habitat, abiotic factors such as currents (that bend blades and affect swimming ability), turbidity (that influences detection ranges for predators and prey), water depth, temperature, and proximity to alternative patches and habitats (that allow prey an alternative means of avoiding predators) may interact with structural complexity and prey density to mediate predator-prey interactions. The presence of higher-order predators that represent a threat to mesopredators such as kelpfish also may strongly influence foraging behaviors and survival rates of epifaunal prey. Further research on the effects of structural complexity in marine systems and elsewhere should consider how factors that covary with

habitat structure may add complexity to simple relationships between habitat structure and predation.

8: Part C: a mathematical model for eelgrass nursery habitat

Rationale

Individual experiments on the abundance and behavior of fishes and their invertebrate prey have revealed a wealth of information about how eelgrass habitat structure can influence patterns of abundance and processes such as predator-prey interactions. However, a further question is: how do these individual interactions and patterns, which operate at small temporal and spatial scales, scale up to the level of the eelgrass landscape? In other words, how do the choices made by organisms interact with habitat structure to dictate eelgrass nursery habitat function? Mathematical modeling is a tool that can be used to answer this kind of question. We created a computer model in which mesopredatory organisms inhabiting an eelgrass habitat interact with their prey (which they hunt) and their predators (which they try to avoid). A primary question being tested with the model is how fragmenting the eelgrass habitat influences the function of eelgrass as a nursery for mesopredators. Likewise, we also varied the relative amount of high complexity eelgrass in the habitat to test how processes in a sparse eelgrass bed might differ from those in a dense eelgrass bed. The organisms interacting in the model eelgrass habitat can be programmed with different abilities to move, find prey, and detect predators, and we also varied their habitat preferences. Thus, we were able to test how (e.g.) preference for high complexity eelgrass by kelpfish would affect their ability to avoid predators and capture prey, based on their ability to detect other organisms and the distribution of prey that we measured in the field (see objective 1). This level of “success” that mesopredators have then was compared to that from other scenarios in which they had different preferences and their prey had an alternate distribution. The result was a prediction about how choices made by organisms interact with eelgrass habitat structure to dictate eelgrass nursery habitat value.

Methods-in-brief: To address objective 3, we used NetLogo software to create an interactive, individual-based computer model in which simulated prey and predator species interact within an eelgrass landscape. NetLogo is free software available online (<http://ccl.northwestern.edu/netlogo/>). In the interactive model, users can construct different simulated eelgrass landscapes that vary in their patchiness and level of structural complexity (**Figure 13**). Landscape structure in the model was based on digital mapping of eelgrass habitat using a scientific echosounder in consultation with Dr. Kwang-Young Kim of Chonham University in Korea, who was a visiting researcher at SDSU during the time of our study (see **Figure 2**). Users also can vary the density of prey and their relative habitat preferences as well as their search and capture capabilities. The preferences exhibited by organisms are created by defining probabilities that they will perform certain actions when confronted with various

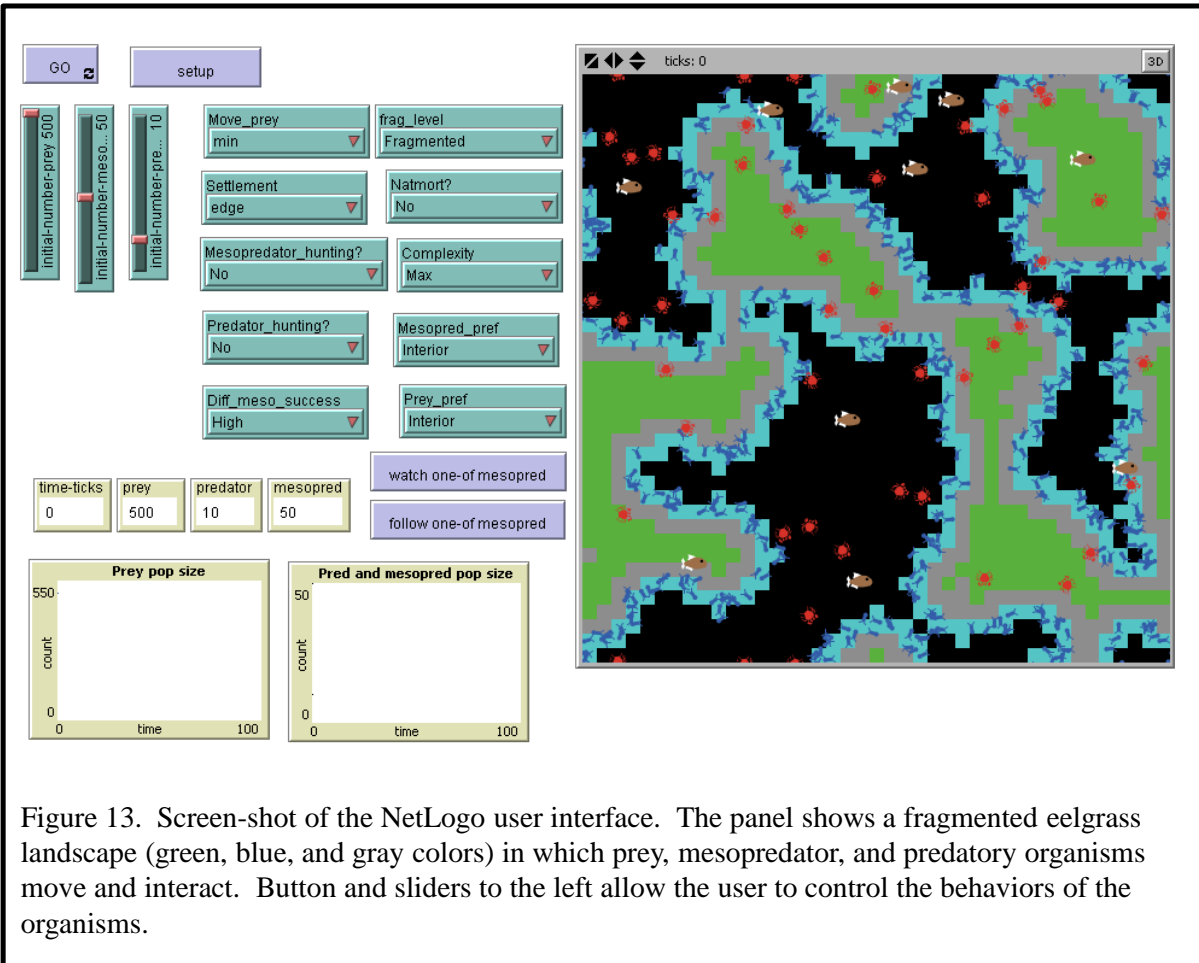


Figure 13. Screen-shot of the NetLogo user interface. The panel shows a fragmented eelgrass landscape (green, blue, and gray colors) in which prey, mesopredator, and predatory organisms move and interact. Button and sliders to the left allow the user to control the behaviors of the organisms.

scenarios. For example, when a mesopredator is confronted with a predator, it is likely to take a particular action (e.g. “run away”) in one habitat type (e.g. sparse eelgrass), but take another action (e.g. “hide”) in a different habitat type (e.g. dense eelgrass).

After creating the model, we ran simulations that calculated overall mesopredator success (a combination of survival rate and the intake of prey over a given amount of time) given different combinations of factors. The factors varied were:

- eelgrass fragmentation level (continuous vs. fragmented landscapes);
- relative amount of high complexity eelgrass (eelgrass landscape was relatively sparse or dense);
- prey preference for habitat (prey could prefer habitat edges (like amphipods) or interiors (like grass shrimp; see objective 1));
- mesopredator preference for habitat (mesopredators could prefer to hunt along patch edges or within patch interiors)

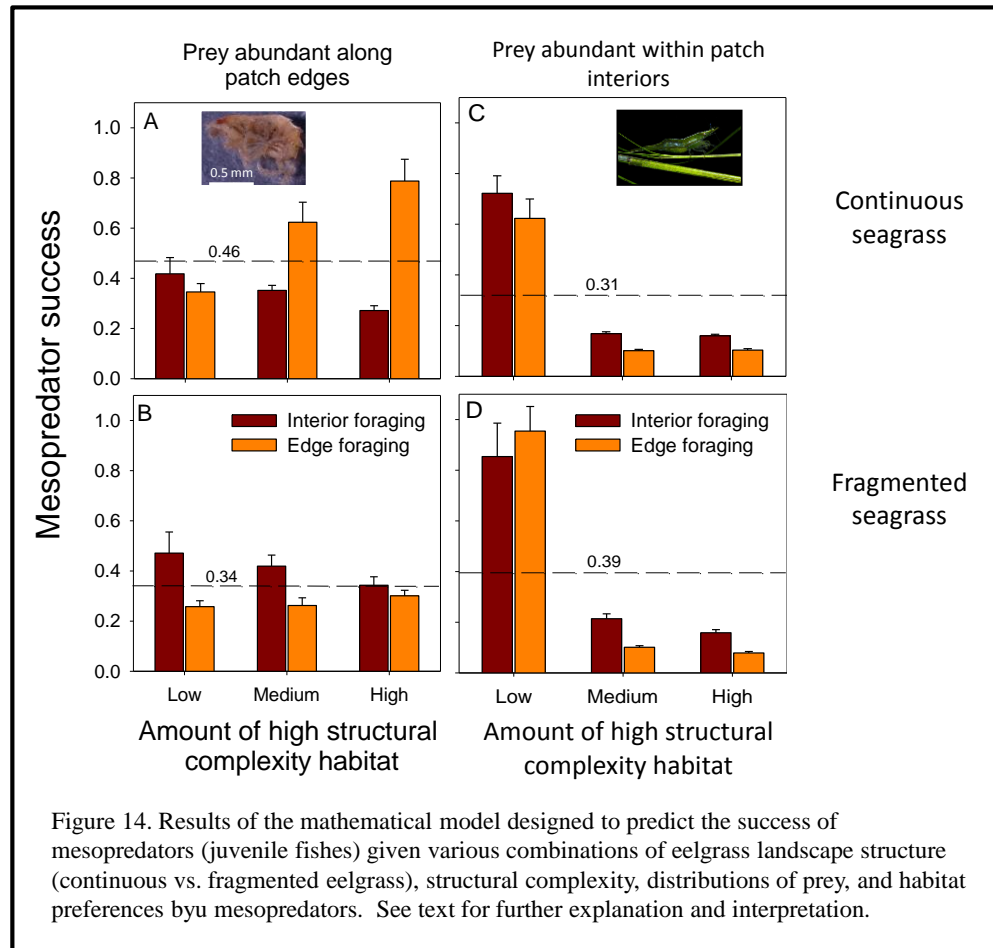
Results and discussion: Overall, the model revealed that juvenile fishes should be most successful (success = a combination of surviving predatory attacks and consuming prey) when (i) eelgrass is continuous, (ii) prey are most abundant along patch edges, (iii) juvenile fishes prefer patch edges, and (iv) there is an abundance of high complexity eelgrass in the landscape. Under this scenario, juvenile fishes are able to take advantage of the safety of high density eelgrass to hide from predators, but also are able to hunt efficiently in low density eelgrass along patch edges where their relative success rates at capturing prey are high. Results were very different when juvenile fishes preferred the interior of eelgrass beds for foraging, but their prey still preferred patch edges. In this scenario, having more high complexity eelgrass in the landscape actually reduced fish success, because they were inefficient at hunting within patch interiors due to low prey densities, although they survived at a high rate. In contrast, when eelgrass was continuous and prey and fishes both preferred patch interiors, success rate was maximized when eelgrass complexity was low. In this case, juvenile fishes had ample room to flee from

predators, which they could detect at a distance, but also had relatively high rates of prey intake due to high efficiency in low density eelgrass.

Not surprisingly, juvenile fishes experienced reduce success overall in fragmented eelgrass habitat, primarily because they were more vulnerable to large predatory fishes (**Figure 14**).

However, they did have relatively high success in fragmented eelgrass

when the complexity of eelgrass was low throughout the landscape, though only when prey preferred patch interiors. This was surprising because the combination of fragmented eelgrass and low complexity eelgrass was expected to make juvenile fishes very vulnerable to predators.



If juvenile fishes remain within the relatively safety of patch interiors, however, they can avoid many predatory attacks and simultaneously be efficient at capturing prey. Juvenile fish success was not high when prey preferred patch edges, because juvenile fish were drawn to patch edges to forage where they were vulnerable to larger predators, which significantly reduced survival rates.

The modeling portion of the study produced expected and unexpected results. We expected, and found, that fragmenting eelgrass, which produces more edges and can reduce overall levels of complexity, resulted in lower mesopredator success overall. We did not expect however, that mesopredator success would be relatively high in fragmented eelgrass that was low in complexity. This was the case only when prey preferred patch interiors. The field experiments from objective 1 revealed that grass shrimp, a primary prey item for mesopredators like kelpfish, are most abundant within patch interiors. We would therefore predict from the model that the proportion of the diet of juvenile kelpfish composed of grass shrimp would increase when eelgrass is fragmented and relatively sparse. This prediction can be tested in further experimentation in naturally occurring eelgrass or in laboratory mesocosms in which simulated landscapes are created for kelpfish and their prey.

Overall, the model revealed that it is very important to consider the behavior of animals when attempting to predict the consequences of changes to eelgrass habitat to the animals that inhabit it. For instance, the value of eelgrass as a nursery habitat can be high even when eelgrass is fragmented and is sparse, but only when organisms are displaying particular preferences for habitat. We also note that though our model uses information from field and laboratory studies, it still only encompasses information from two species, kelpfish and grass shrimp. More behavioral data should be collected on other mesopredator and prey species to test the generality of the model for overall eelgrass nursery habitat function.

9: Part D: outreach

We have conducted a variety of exercises with the Ocean Discovery Institute (formerly known as Aquatic Adventures) to educate underrepresented youth about marine ecology, the value of marine habitats, and mathematical modeling. Ocean Discovery Institute conducts afterschool programs and study abroad programs in marine biology and ecology for K – 12 students from the San Diego area. First, on two occasions, approximately 15 Ocean Discovery Institute students (6th – 8th grade) visited the Coastal and Marine Institute Laboratory to conduct an experiment examining how habitat influences predator-prey interactions. This simple experiment quantified how the efficiency of hunting for small fishes changes with the addition of habitat structure into experimental arenas. Students were introduced to the concepts and to experimental design, and they then ran the experiment and observed fish hunting shrimp prey within aquaria containing simulated eelgrass vs. aquaria with no eelgrass. Working with us, the students then calculated some simple statistics showing how predation efficiency varied with the addition of habitat structure to aquaria.

Second, we conducted two instructional sessions in the SDSU Biology Department's computer laboratory, in which approximately thirty 6th – 8th grade students used our mathematical model to perform an experiment on predator-prey interactions in simulated eelgrass habitat. The students were given a short lecture to introduce the concept and utility of modeling, and they then followed instructions to build their own mathematical model in NetLogo. This simple model allowed the students to create a world in which predator and prey organisms interact, and in which habitat can be added or removed to act as a protective area for prey. Finally, the students used our full model for eelgrass habitat to test how changing the behaviors of the organisms influenced the effectiveness of eelgrass nursery habitat. After the students ran simulations, we discussed the outcomes as a group and created simple graphs to indicate how changing eelgrass habitat structure influenced nursery habitat effectiveness.

10. Outcomes: theses, presentations, and papers resulting from the project

Part A

Part A was the basis for a master's thesis by graduate student Eliza Moore, who graduated in 2009. The study was published in the peer-reviewed, widely-read journal *Oikos* in 2010.

Peer-reviewed papers:

Moore, E.C. and K.A. Hovel. 2010. Relative influence of habitat complexity and proximity to patch edges on seagrass epifaunal communities. *Oikos* 119: 1299-1311.

Conference presentations:

Moore, E. and K.A. Hovel. 2008. Relative influence of habitat complexity and edges on seagrass epifaunal communities. Western Society of Naturalists 89th annual meeting, Vancouver, BC, Canada.

Moore, E. and K.A. Hovel. 2007. Seagrass habitat structure: relative effects of structural complexity and location within patches on epifaunal abundance and diversity. Western Society of Naturalists 88th annual meeting, Ventura, CA.

Moore, E. and K.A. Hovel. 2007. Seagrass habitat structure: relative effects of structural complexity and location within patches on epifaunal abundance and diversity. Benthic Ecology Meeting, Atlanta, GA.

Part B

Study #1 of Part B was the basis for a master's thesis by SDSU graduate student Kelly Tait, who graduated in 2011. Study #2 of Part B was the basis for an undergraduate honors thesis by SDSU student Rachel Lannin, who graduated in 2010. Follow-up experiments on Part

B also resulted in independent study projects for SDSU undergraduate students Stacey Virtue, Alex Warneke, and Alterra Sanchez.

Peer-reviewed papers:

Lannin, R. and K.A. Hovel. In press. Variable prey density modifies the effects of seagrass habitat structure on predator-prey interactions. Marine Ecology Progress Series.

Conference presentations:

Virtue, S. and K.A. Hovel. 2011. Seagrass nursery habitat function: relative effects of habitat structure and prey density on mesopredators foraging efficiency. Benthic Ecology Meeting, Mobile, AL.

Warneke, A., Virtue, S., Sanchez, A., and K.A. Hovel. 2011. Seagrass nursery habitat function: relative effects of habitat structure and prey density on mesopredator foraging efficiency. San Diego State University Student Research Symposium, San Diego, CA.

Tait, K. and K.A. Hovel. 2011. Relative effects of structural complexity, predation risk, and food availability on habitat selection of seagrass fauna. Benthic Ecology Meeting, Mobile, AL.

Hovel, K.A. 2011. Habitat edges in marine systems: their role in shaping pattern and process. Benthic Ecology Meeting, Mobile, AL.

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Lannin, R. and K.A. Hovel. 2009. Variable prey density modifies the effects of seagrass habitat structure on predator-prey interactions. San Diego State University Student Research Symposium, San Diego, CA.

Part C

Conference presentations:

Hovel, K.A. and H.M. Regan. 2010. Marine habitat structure and predator-prey interactions: integrating effects of landscape structure and structural complexity using an individual-based, spatially explicit model. Benthic Ecology Meeting, Wilmington, NC.

Hovel, K.A. and H.M. Regan. 2009. Using an individual-based model to assess nursery habitat function. Coastal and Estuarine Research Federation, Biannual meeting, Portland, OR.

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12. Appendix 1: Full methods for Part A.

Our study was conducted in the eelgrass (*Zostera marina*) beds of San Diego Bay, California, USA (32° 44' N x 117° 10' W) during the summer of 2007. San Diego Bay is a heavily developed urban estuary used for shipping, military operations, and recreation. Freshwater inflow is low, and hydrodynamic conditions are largely tidally driven (Largier et al. 1997). The front region of the bay is characterized by relatively low water residence times, moderate currents, and low fluctuations in salinity and temperature through the year, whereas higher water residence time in the southern or back region leads to slower currents and greater fluctuations of salinity and temperature. We conducted surveys of the eelgrass, epifauna, and fishes at two sites located in the central region of the bay (south central bay (SCB), and north central bay (NCB)) and one site close to the bay mouth (Shelter Island (SI)) during June and August of 2007. We also conducted a manipulative experiment to address the relative influences of structural complexity, distance from the edge, and predator presence on epifaunal communities at SI. At all sites eelgrass patches are 20-30 m in width and run parallel to shore for approximately 250 m. Surveys and experiments were conducted at depths of 2-5 m below mean lower low water (MLLW).

2.1 Eelgrass, epifauna, and fish surveys

To determine whether eelgrass structural complexity (shoot density, shoot length, and biomass per unit area) varied with distance from the edge within eelgrass patches at each site, we collected samples at the outer edge (vegetated substrate at the bayward sand-eelgrass interface), the inner edge (vegetated substrate, 1 m from interface), and within the interior (vegetated substrate, 5 m from interface). Eelgrass cores (12 cm diameter x 15 cm depth) were taken every 5 m along 40 m transects at each of the three distances running parallel to the edge of the bed (n = 8). We counted shoots and determined mean shoot length per core by averaging the length of the longest blade per shoot. Shoots were separated from rhizome material and dried at 60° C to a constant weight for estimations of aboveground biomass. We used a multivariate analysis of variance (MANOVA) to test whether the combined eelgrass structural complexity variables (shoot density, length, and biomass) vary with distance from the edge, site, and sampling period. We followed MANOVAs with separate analyses of variance (ANOVAs) for each dependent variable. For these and all other ANOVAs, we calculated the proportion of variance accounted for by each factor and tested for differences in means using Student-Newman-Keuls (SNK) multiple comparisons. Before analyses were performed, we visually inspected the data for normality and we tested for homogeneity of variances using Cochran's test, transforming the data where necessary.

We sampled for mobile epifauna along each transect by capturing organisms within a small underwater sieve made of 20 cm diameter x 25 cm tall PVC pipe with a mesh bag attached to one end (mesh size ~400 µm). Every 5 m along each transect (away from where eelgrass cores were taken) divers quickly slipped sieves over eelgrass shoots, cut shoots at the sediment surface

using scissors, and then placed a 500 μm screen under the sieve to capture the contents. Contents were stored on ice before transport to the lab where shoots were rinsed in freshwater and shaken to release epifauna which were frozen for preservation. Epifaunal samples were sorted and individuals in each taxon were counted to obtain densities. Estimates of total biomass per sample for six epifaunal categories (fishes, gastropods, crabs, shrimp, peracarid crustaceans, and ostracods/copepods) were obtained after drying epifauna in an oven at 60° C for 48 h. Soft-bodied epifauna (including nematodes and polychaetes) were excluded from analysis due to poor preservation in frozen samples and their relatively low abundance in fish stomachs (see Results). We used ANOVAs to test for the effects of distance from the edge, site, and sampling periods on total epifaunal abundance, species richness, Simpson's index of diversity (D_s), and the biomass of selected epifaunal species that dominated fish stomach contents.

We used non-metric Multi Dimensional Scaling (nMDS) to investigate patterns of epifaunal community composition between sites and among the three distances from the edge for each sampling period. nMDS analyses were conducted on a matrix of Bray-Curtis similarities based on square-root transformed abundances of all taxa to reduce the influence of a few, highly abundant taxa (Clarke and Warwick 2001). Where data were shown to cluster in nMDS plots, ordination was followed by analyses of similarity (ANOSIM) to further investigate the causes of those patterns. ANOSIM results are shown where pair-wise comparisons had R-values greater than 0.60, indicating strong group differences. The R-statistic is considered more robust to sample-size influence than traditional p -values.

To quantify the density and diversity of fishes at the edge and interior of eelgrass patches at each site, we towed a non-destructive beam trawl (1.5 m wide x 0.6 m tall, 6 mm mesh cod end) at a speed of 3 knots for 100 m against tidal flow through the beds in June and September 2007. The precision of the trawl did not allow us to distinguish outer from inner edge, and we thus collected samples within the patch interior and within 2 m of the eelgrass-unvegetated sediment interface. Three trawls were conducted at the edge and three in the interior at each site in each of the two sampling periods. Fishes captured in trawls were identified to species, measured (fork length (FL)) on the boat, and then released. A subset of fish from each species at each site was immediately frozen and later dissected for stomach content analysis during each sampling period. All identifiable organisms in the fish stomachs were processed as described for epifaunal sampling above. We used separate ANOVAs to test for differences in fish abundance, species richness, D_s , and mean FL between distance from patch edge, site, and sampling period, and conducted nMDS as described above to test for variability in community composition. We also explored variation in stomach content composition using nMDS and ANOSIM as above. Fish with no identifiable stomach contents were excluded from that analysis.

2.2 Caging experiment

We conducted a manipulative experiment using artificial seagrass units (ASUs) at SI in July 2007 to determine the relative effects of structural complexity, distance from the edge, and

exposure to predators on the density and diversity of epifauna. ASUs are widely used to control structural complexity in seagrass experiments and are colonized by epifauna similarly to living seagrass. ASUs consisted of green polypropylene ribbon to simulate eelgrass blades tied to a mesh base (25 cm x 25 cm) anchored to the sediment. ASUs were placed 5 m apart along the edge of the eelgrass patch (within 0.5 m of the sand-eelgrass interface) and in the interior of the patch (> 5 m from the sand-eelgrass interface) at depths of ca. 3 – 4 m MLLW. Each ASU was one of two simulated shoot densities: sparse (150 shoots m⁻²) or dense (600 shoots m⁻²), representing extremes observed at this site. Exposure to predators was varied by enclosing a subset of ASUs within predator-exclusion cages, while leaving a second subset open to predators (no cage) and a third subset enclosed within partial cage-controls (N = 5 ASUs/caging treatment/complexity treatment/distance from the edge * 3 caging treatments * 2 complexity treatments * 2 distances = 60 ASUs). Cages enclosed the entire ASU and were 71 cm in height with a mesh size of 6 mm to exclude fish predators and allow colonization by epifauna. Cage-controls were partial cages with two open walls, and controlled for potential caging artifacts which may include reduced flow, shading, and addition of structure to the habitat. ASUs were deployed for 28 days, and we cleaned the mesh on all cages and partial cages weekly. At the end of deployment ASUs were collected by slipping a mesh bag over the whole unit (after gentle removal of caging structure where present). On shore, the mesh bags and ASUs were rinsed with freshwater through sieves (500 µm) and all epifauna collected were frozen and processed as above. We used separate three-way ANOVAs followed by SNK multiple comparisons to test whether total epifauna abundance, species richness, D_s, and prey biomass varied with location within the patch, complexity, and exposure to predators. We again utilized nMDS to compare differences in community composition between treatments.

13. Appendix 2: Full methods for Part B, study #1

Study species

Hippolyte californiensis (Crustacea: Decapoda: Hippolytidae) ranges from the coast of Alaska to the Gulf of California where it prefers eelgrass habitat and primarily consumes epiphytic algae growing on eelgrass blades. *Heterostichus rostratus* (Chordata: Perciformes: Clinidae; hereafter “kelpfish”) ranges from British Columbia to Cabo San Lucas, Baja California, Mexico. Juvenile kelpfish commonly form aggregations in eelgrass (*Zostera marina*) habitat before transitioning to a solitary lifestyle in coastal kelp forests. Their diet consists primarily of small crustaceans, with grass shrimp composing up to 20% of the biomass in kelpfish guts in San Diego Bay (Moore and Hovel 2010). Both species are abundant in San Diego Bay eelgrass habitat, reaching densities of ca. 2 and 2000 individuals m⁻² for kelpfish and grass shrimp, respectively.

We collected grass shrimp and kelpfish by dipnetting and seining in eelgrass beds bordering Shelter Island in San Diego Bay, CA. Animals were held for no longer than one week in recirculating seawater tanks at the San Diego State University Coastal and Marine Institute

Laboratory before being used in experiments. Grass shrimp were fed by supplying them with eelgrass blades containing epiphytic algae, and kelpfish were fed grass shrimp *ad libitum*. Each individual was used only once in experiments.

General experimental design

We tested how predation risk and food availability influenced habitat selection for our two study species following the protocol described in Olabarria et al. (2002) and Underwood et al. (2004) in which behavior is compared between treatments in which animals are presented with a choice and treatments in which there is no choice. In this design, active selection for habitat is noted when organisms are more selective in treatments that offer a choice of habitats than in treatments that offer no choice. Habitat selection was measured by observing the behavior of grass shrimp and kelpfish within four outdoor mesocosms containing simulated eelgrass habitat. Each 2.18 m diameter x 0.91 m high mesocosm contained two artificial seagrass units (ASUs) that were embedded within 10 cm of beach sand, and filled with flow-through seawater to a depth of 50 cm. ASU's consisted of 40 cm long x 0.48 cm wide simulated eelgrass shoots made of polypropylene ribbon tied to a base made of 4 mm x 4 mm Vexar mesh. Each ASU was the size and shape of one half the mesocosm, resulting in complete simulated eelgrass coverage within mesocosms. Two levels of structural complexity were used in all experiments: low shoot density (= 200 shoots m⁻²) and high shoot density (= 800 shoots m⁻²). We chose these two simulated shoot densities to represent relatively low and high values found within naturally occurring eelgrass beds in San Diego Bay. For each experiment, two of the four mesocosms (treatments 1 and 2) offered organisms a choice of low or high complexity eelgrass (a low complexity ASU on one side and a high complexity ASU on the other), and the other two mesocosms offered organisms no choice (treatment 3: high shoot density ASUs on both sides; treatment 4: low shoot density ASUs on both sides; Figure 1). Treatments were randomly assigned to the four mesocosms and a coin flip determined which side of the mesocosm each ASU would occupy. Treatments were re-randomized between trials and experiments. The basic experimental design calls for placing animals on one side of each mesocosm (Table 1) and then quantifying the proportion of individuals that chose to leave or that remained on that side in the presence or absence of choice. Selection for a particular treatment is established when the proportion of individuals found within that treatment is greater when they had a choice of habitats than what would be predicted from their distribution in the absence of choice (Olabarria et al. 2002).

Experiment 1: selection for habitat structural complexity

We performed experiment 1 to test whether grass shrimp and kelpfish select habitat based only on structural complexity. Previous field surveys and experiments in San Diego Bay indicated a strong and weak preference for high complexity eelgrass over low complexity eelgrass for grass shrimp and kelpfish, respectively. The experiment was performed separately

for each species. To perform experiment 1 for grass shrimp, on the morning of an experimental trial, 100 shrimp (density = 26.8 m^{-2}) were released on the appropriate side of the mesocosm in each of the four treatments (Table 1). Shrimp were then allowed to move throughout the mesocosm undisturbed for 24 h. We chose this time frame to include both diurnal and nocturnal periods, as grass shrimp may be more active at night than during the day. Pilot experiments revealed that results did not differ between 24 and 48 h time frames, suggesting that 24 h was enough time for grass shrimp to select habitat. After 24 h, a partition made of weighted window screening was placed directly in the center of each mesocosm to restrict any further movement between sides, and each side of each mesocosm was vigorously dipnetted to recover all grass shrimp. Dipnetting was highly effective at recovering grass shrimp, generating a 100% recapture rate both in high and low complexity ASUs in pilot experiments. We conducted 10 trials of this experiment with grass shrimp between 12 January - 29 January 2010.

To perform experiment 1 with kelpfish, between the hours of 0900 and 1500 five kelpfish (density= 1.59 m^{-2} , mean fork length (FL) = $116.9 \pm 2.6 \text{ mm SE}$) were released into the appropriate side of the mesocosm in each of the four treatments (Table 1). Following a 15 min acclimation period, two observers recorded the amount of time each fish spent on each side of mesocosms for one hour. Observers also recorded general observations of fish swimming behavior, including the number of times each individual crossed from one side of a mesocosm to the other. Individual fish were distinguished based on unique colorations and markings. At the end of an hour, we placed a partition through the center of each mesocosm to restrict any further movement and all fish were recaptured using a dipnet. Fish then were released back into San Diego Bay. We conducted 5 trials of this experiment with kelpfish between 11 May - 7 June 2010.

To analyze data on grass shrimp and kelpfish habitat preference, we first used separate one-way analyses of variance (ANOVAs) to determine whether (1) the proportion of shrimp that remained on the starting side after 24 h, or (2) the total proportion of time spent in the starting side by kelpfish varied among treatments. Before running ANOVAs we tested for homogeneity of variances using Cochran's test and used normal probability plots to test for normality, and transformed data when necessary to meet the assumptions of ANOVA in this and all subsequent tests (Underwood 1997). If ANOVAs revealed a significant effect of treatment on the dependent variable, we tested for differences among individual treatments using *a priori* contrasts (Sogard and Olla 1993, Olabarria et al. 2002; Table 2). We selected *a priori* contrasts over post-hoc multiple comparisons because our experimental protocol calls for testing for differences between specific groups of means (as opposed to testing every mean against every other mean). *A priori* contrasts increase statistical power by reducing the number of tests between means and by testing for specific hypotheses that are formed before experiments are conducted (Underwood 1997). Two sets of *a priori* contrasts were relevant for our experimental design: set 1 tested for preference for high complexity eelgrass, and set two tested for preference for low complexity

eelgrass. Specific comparisons for each set of contrasts are listed in Table 2 and were used for both species and in all experiments.

Experiment 2: complexity and predatory threat

In experiment 1, there was a weak but non-significant preference for high complexity eelgrass by grass shrimp, and a strong preference for high complexity eelgrass by kelpfish (see *Results* below). In experiment 2, we added predators to the high complexity habitat of mesocosms to determine whether animals prefer low complexity eelgrass when a predatory threat is present in high complexity eelgrass. For each species, trials were identical to experiment 1 except that caged predators were added to high complexity eelgrass wherever it occurred among the four mesocosms (Figure 1, Table 1). Housing predators in cages allowed them to be chemically and visually detected but restricted them from consuming animals and from moving to the other side of mesocosms (Holbrook and Schmitt 1988). For grass shrimp, we added a predatory threat by placing one juvenile giant kelpfish (80-160 mm FL) in each of three rectangular clear plastic mesh cages (20 cm x 10 cm x 10 cm; mesh size 4 mm x 4 mm). Three empty cages were added to low complexity eelgrass to control for added structure. After 24 h, all six cages were removed and mesocosms were sampled between the hours of 1100 and 1400 as described in experiment 1 above. We conducted 10 trials of this experiment with grass shrimp between 9 February - 30 April 2010.

For kelpfish, we added a predatory threat to high complexity eelgrass by placing one spotted sand bass (*Paralabrax maculatofasciatus*; 230-280 mm total length) into each of two 40 cm x 12 cm x 12 cm plastic mesh cages (4 mm x 4 mm mesh size). Two empty cages were placed in the low complexity side of mesocosms to control for added structure and trials were conducted as described for experiment 1 above. We conducted five trials of this experiment with kelpfish between 11 June - 29 June 2010.

Experiment 3: complexity and food availability

In experiment 3 we tested whether grass shrimp and kelpfish select low complexity eelgrass over high complexity eelgrass when food is available only in low complexity eelgrass and predators are absent. To provide food for grass shrimp, we allowed all ASUs to become fouled with epiphytic algae from the flow through seawater system (soak time = 2 wk), and then scrubbed algae from all high complexity ASUs. Trials (n = 10) were conducted as described above between 10 September - 13 October 2010. For kelpfish, we added food to low complexity eelgrass by releasing 100 grass shrimp into low complexity ASUs in each mesocosm. We placed a partition between ASUs before adding grass shrimp and allowed shrimp to acclimate to ASUs for 25 minutes before gently removing the partition and releasing five kelpfish on the appropriate side of the mesocosm to begin a trial (Table 1). Trials (n = 5) were conducted as described above

between 2 July - 22 July 2010, except that we also quantified the number of grass shrimp found in each ASU to ensure that shrimp remained in low complexity eelgrass over the course of the trials. On average, only 1.5% of grass shrimp moved between ASUs over the 1 h trials.

Experiment 4: complexity, predatory threat, and food availability

Because kelpfish (but not grass shrimp) did not prefer low complexity eelgrass even when predators were present in high complexity eelgrass or food was present in low complexity eelgrass (see *Results* below), we conducted a fourth experiment to determine if kelpfish prefer low complexity eelgrass when both a predatory threat is added to high complexity eelgrass and food is added to low complexity eelgrass. Between 16 December - 14 January 2011, we conducted $n = 5$ trials of experiment 4 by adding caged spotted sand bass to high complexity ASUs and grass shrimp to low complexity ASUs before releasing five kelpfish into the appropriate side of each mesocosm as described in experiments 2 and 3 above. 1

14. Appendix C: Full methods for Part B, study #2

Study species

We chose juvenile *H. rostratus* (hereafter “kelpfish”) and *H. californiensis* (hereafter “grass shrimp”) for our study species due to their high abundance (reaching densities of ca. 2 and 2000 individuals m^{-2} in southern California eelgrass habitat, respectively: Moore & Hovel 2010) and their strong trophic linkage. Though the most abundant epifaunal prey item found in the guts of juvenile giant kelpfish are amphipods, grass shrimp are a common component of the diet, composing up to 20% of the biomass in kelpfish guts. These two species also represent groups of vertebrate mesopredators and invertebrate grazers that may exert strong top-down control on eelgrass growth and abundance via their predator-prey interaction. Kelpfish are active predators that swim slowly within the eelgrass canopy searching for epifaunal prey such as grass shrimp, which are often found clinging to eelgrass blades where they consume epiphytic algae (K. Hovel, personal observation).

We collected kelpfish (80-120 mm fork length (FL)) with a beach seine and collected grass shrimp by dip netting within shallow subtidal eelgrass habitat within San Diego Bay, California. Collections were performed frequently throughout the experimental period to prevent holding organisms for more than 10 d and so that we did not have to reuse animals in the experiments. Kelpfish and grass shrimp were held in a recirculating seawater system at the SDSU Coastal and Marine Institute Laboratory in San Diego before use in experiments. Kelpfish were fed grass shrimp *ad libitum* for six days while in captivity and were starved for 2 d before being used in trials. Water temperature was held constant at 20 °C for the experiments.

Experimental design and procedure

We conducted experiments within glass mesocosms (122 x 61 x 61 cm) filled to a depth of 8 cm with clean beach sand and then filled with recirculating seawater to a depth of 45 cm. We varied structural complexity within mesocosms by transplanting eelgrass from San Diego Bay to create six shoot densities, each randomly assigned to one mesocosm: 20, 40, 80, 160, 250, or 320 shoots m^{-2} . Our chosen shoot densities are relatively low for many eelgrass habitats worldwide, and it was necessary to cap our range of shoot densities at 320 m^{-2} because it was very difficult to observe grass shrimp at higher shoot densities in preliminary trials. Our results and conclusions thus apply only to relatively low levels of eelgrass habitat structure. To examine the relative effects of varying prey density and varying structural complexity on prey survival, encounter rates, foraging behavior of predators, and predator avoidance behavior by prey, we conducted two experiments, one in which grass shrimp density was held constant across the six levels of structural complexity, and one in which grass shrimp density increased with structural complexity. We used a density of 20 shrimp per mesocosm in the constant prey density experiment, and in the variable prey density experiment we increased shrimp density proportionally with shoot density to maintain an approximate ratio of one grass shrimp for every 2-3 shoots (Table 1). Our prey densities are comparable to those occurring at our collection site in San Diego Bay, and using these prey density levels allowed us to recover 100% of grass shrimp at the conclusion of trials with minimum disturbance to transplanted eelgrass.

Before transplantation, intact eelgrass shoots were rinsed to remove sediment, macroalgae, and large organisms, and then were soaked in freshwater for 30 min to remove remaining epifauna from the blades. Shoots were added to mesocosms by tying rhizomes to randomly selected points on a plastic grid buried beneath the sediment. Blades then were trimmed to be flush with the water's surface. Shoots began to decay after approximately two weeks, at which point mesocosms were randomly assigned a new shoot density treatment and all shoots within each mesocosm were replaced with fresh shoots from San Diego Bay. To minimize disturbance by observers during predation trials, we covered three sides with opaque white plastic sheeting, and placed a mesh blind over the remaining side. Mesocosms were illuminated by two fluorescent Coralife[®] aquarium bulbs during trials.

Prior to the start of a trial, the experimental tanks were cleaned of any eelgrass detritus and detached eelgrass blades, and replacement shoots, if necessary, were haphazardly placed in mesocosms. Grass shrimp (12 – 20 mm total length) then were added to an aquarium and were allowed to acclimate for 30 min, after which time the FL of one kelpfish was measured and the kelpfish was placed in a plastic mesh container floating within the mesocosm. After 15 min of acclimation to the mesocosm the kelpfish was released by gently inverting the basket which began a trial. For each 75 min trial, one observer recorded kelpfish behaviors and one observer recorded grass shrimp behaviors using voice recorders (see *Predator and prey behaviors* below). After 75 min, we removed kelpfish from mesocosms and collected all surviving shrimp within

mesocosms by dipnetting for 15 min, which was 100% effective at recovering grass shrimp in pilot experiments using up to 80 grass shrimp and no kelpfish. At the conclusion of each trial, kelpfish were placed in plastic containers without eelgrass in which they were offered an additional five “post-trial” grass shrimp for 10 min, after which time we counted the number of post-trial grass shrimp consumed. This was done to determine whether grass shrimp survival rates and kelpfish behaviors could be influenced by satiation as well as structural complexity and prey density, particularly at low levels of structural complexity or high levels of prey density (see *Statistical analysis* below). We conducted four replicate trials for each structural complexity treatment in the constant shrimp density treatment ($n = 24$), and six replicate trials for each shoot density in the variable shrimp density treatment ($n = 36$) between March and October 2009.

Predator and prey behaviors

We quantified several distinct components of predator-prey interactions that affect predator foraging efficiency and that may be influenced by structural complexity or prey density (Ryer 1988; Table 2). First, we identified a predator-prey encounter as an obvious fixation of both eyes of the kelpfish on a grass shrimp, accompanied by a halt in swimming (sensu Ryer 1988). Thus, an encounter involves an obvious detection of prey by kelpfish, but would not include a “passive” detection made without an obvious change in kelpfish behavior, which we would not be able to identify. In addition to counting the number of encounters per trial, we divided the number of encounters by the starting number of prey to serve as a measure of the probability that an individual grass shrimp would encounter a kelpfish. Second, after detecting prey, predators must choose whether to attack, and attacks may or may not be successful. We therefore calculated the proportion of encounters that resulted in attacks by kelpfish and the proportion of attacks that were successful. Though we also counted the number of unsuccessful attacks that were followed by a pursuit of prey by kelpfish, there were too few pursuits to conduct statistical analysis. Third, we quantified the total number of seconds kelpfish spent in motion, and calculated proportional kelpfish activity by dividing the number of seconds spent in motion by the total number of seconds in a trial (4500). High rates of activity suggest predators are engaging in active hunting, whereas low rates of activity suggest a sit-and-pursue strategy (Savino & Stein 1989, Michel & Adams 2009). Finally, predator foraging efficiency also may depend on the tendency of prey to detect predators and attempt to flee from them, and we therefore calculated the proportion of encounters that resulted in escape attempts by grass shrimp prey (before an attack or after an unsuccessful attack), and the number of times shrimp switched sides of eelgrass blades (“side switching”). Side switching was used by the grass shrimp *Tozeuma carolinense* to avoid being detected or pursued by predators (Main 1987) and we observed *H. californiensis* performing this behavior in pilot experiments. To record side switching, we haphazardly selected individual shrimp to observe for 5 min intervals, during which we recorded the number of times an individual moved to the other side of an eelgrass

blade. The total number of times side switching was observed during a trial was divided by the number of shrimp observed (11 per trial on average) to yield an average frequency per five minute interval.

Statistical analysis

For the analysis of grass shrimp proportional survival, we first determined whether possible satiation of the kelpfish at low levels of structural complexity or at high levels of prey density was influencing apparent effects of structural complexity and prey density on prey survival rates. To do this we used linear regressions to determine whether the number of post-trial grass shrimp consumed by kelpfish varied with eelgrass shoot density in the constant prey density experiment, and with grass shrimp density in the variable prey density experiment. We found no relationship between structural complexity and the number of post-trial grass shrimp consumed by kelpfish in the constant prey density experiment ($df = 1, 22, F = 2.2, P = 0.14, r^2 = 0.05$). For the variable prey density experiment, kelpfish consumed at least some of the offered post-trial grass shrimp at all prey density levels, but the total number of post-trial grass shrimp consumed decreased with grass shrimp density ($df = 1, 32, F = 12.1, P = 0.001, r^2 = 0.25$). To ensure that this did not result in artificially inflated rates of survival at high levels of grass shrimp density, we calculated an adjusted grass shrimp proportional survival for our two highest density treatments by dividing the number of surviving grass shrimp by the maximum number of shrimp a fish was observed to eat over 75 min in pilot experiments (= 49 shrimp). All other shrimp densities were below the maximum number that kelpfish were observed to eat, so for those densities grass shrimp proportional survival was calculated by dividing the number of surviving grass shrimp by the starting number of shrimp.

We used linear regression, non-linear regression, and a comparison of slopes procedure to test whether the influence of structural complexity on grass shrimp proportional survival, as well as all behavioral variables, differed between the two grass shrimp experiments (constant vs. variable prey density). We originally included fish fork length as an independent variable in our analyses, but removed it from final analyses as did not contribute significantly to statistical models. For each dependent variable (Table 2), we first ran separate least-squares linear regressions to obtain best-fit lines and residuals for each experiment. If data appeared strongly heteroscedastic across structural complexity treatments and included one or more extreme outliers (i.e. standardized residual ≥ 3.0), we used least-trimmed squares robust regression rather than linear regression to generate best-fit lines. If upon visual inspection residuals from both regressions were random, we then tested whether effects of structural complexity on the dependent variable differed between the constant and variable shrimp treatments using a *t*-test for equality of slopes. If the residuals from one or both initial regressions appeared non-random, we used quadratic regression to test for a non-linear relationship between the dependent variable and eelgrass structural complexity. We assumed the relationship to be non-linear if the quadratic

regression was significant and explained a larger proportion of the variance in the dependent variable than did the linear regression.

Several outcomes were possible for each dependent variable examined in our experiments. First, significant relationships between structural complexity and the dependent variable may be similar (i.e. have equal slopes) between the two experiments. This would suggest that structural complexity, but not prey density, influences the dependent variable. Second, significant relationships between the dependent variable and structural complexity may be dissimilar between the two experiments, which would suggest that structural complexity influences the dependent variable, but variable prey density modifies this relationship. Third, a significant relationship between the dependent variable and structural complexity may exist only for one of the experiments. For instance, if a relationship between the dependent variable and structural complexity exists only when prey density increases with structural complexity, this would suggest that (i) only prey density influences the dependent variable, or (ii) the effects of structural complexity and prey density are additive. We were not able to distinguish between these two possibilities because we did not vary prey density while standardizing structural complexity. We did not include such an experiment because we were interested in whether variability in prey density would alter the relationship between structural complexity and each dependent variable. Fourth, lack of a significant relationship between the dependent variable and structural complexity in both experiments would suggest that neither structural complexity nor prey density influence the dependent variable.